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***Special Issue***

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***Recovery Potential of Oiled Marine Northern Environments***

Fisheries and Environment Canada
Fisheries and Marine Service

Pêches et Environnement Canada
Service des pêches et de la mer

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## *Editor's Foreword*

For the first time, a sharp integrated focus was brought to bear on world oil pollution at the symposium on "Recovery Potential of Oiled Marine Northern Environments" held at Halifax in October 1977. About 160 scientists from nine countries participated, with particularly strong representation from the Scandinavian countries and with enthusiastic support from scientists from laboratories in other countries, such as the Woods Hole Oceanographic Institution and the National Fisheries Research Center, Seattle.

This special edition of the *Journal* consists of selected papers presented at the symposium. Of particular importance is the paper on the *Torrey Canyon* disaster of 1967 in that it deals with the last remaining ongoing study from the initial oil spill.

The symposium was conceived by Drs John H. Vandermeulen and Patrick D. McTaggart-Cowan in the spring of 1976. By the fall of that year plans were formalized with the originators acting as symposium chairman and cochairman, and Dr D. C. Gordon Jr. joined the team as Program Chairman. Financial support of the Petroleum Association for the Conservation of the Canadian Environment (PACE), the Canadian Department of Transport, and the Canadian Department of Fisheries and the Environment through its Fisheries and Marine Service (Ocean and Aquatic Sciences Directorate) and Environmental Protection Service was instrumental in permitting the symposium to be held and is gratefully acknowledged by all who participated.

Special tribute for the success of the symposium must go to Dr Vandermeulen who was the key figure in conceiving, planning, and carrying out the project. His ingenuity even extended to designing the symposium logo that appears on the cover of this volume. Also, sincere appreciation is extended to Special Editor Natalie Sutterlin who worked hard and long in arranging for the refereeing and in preparing the manuscripts for press.

The *Journal* is privileged to publish this special issue which advances knowledge on the effect of oil pollution on the coasts of the world ocean.

J. C. STEVENSON
*Editor and Director of Scientific Information*

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## *Note du Directeur*

Le symposium consacré au « Potentiel de rétablissement des environnements septentrionaux marins affectés par le pétrole », qui s'est tenu à Halifax en octobre 1977, fut l'occasion, pour la première fois, d'examiner en profondeur le problème mondial de la pollution par le pétrole. Environ 160 scientifiques de neuf pays différents y participèrent. Les pays scandinaves y étaient particulièrement bien représentés, et les scientifiques de laboratoires étrangers tels la Woods Hole Oceanographic Institution et le National Fisheries Center, Seattle, y apportèrent un appui enthousiaste.

Ce numéro spécial du *Journal* contient une sélection d'articles présentés au symposium. L'article sur le désastre du *Torrey Canyon* de 1967 revêt une importance toute particulière parce qu'il traite de la dernière étude en cours qui reste du déversement initial de pétrole.

L'idée du symposium fut conçue par MM. John H. Vandermeulen et Patrick D. McTaggart-Cowan au printemps 1976. Dès l'automne de la même année, les plans en étaient fixés, les initiateurs agissant comme président et co-président du symposium. À l'équipe s'ajoutait M. D. C. Gordon, Jr., comme président du programme. Le symposium reçut l'appui financier de la Petroleum Association for the Conservation of the Canadian Environment (PACE), le ministère des Transports du Canada et le ministère des Pêches et de l'Environnement du Canada, par l'intermédiaire de son Service des pêches et de la mer (Direction générale des sciences océaniques et aquatiques) et de son Service de la protection de l'environnement. Tous les participants expriment leur gratitude envers ces organismes.

Une bonne part du succès du symposium est due à M. Vandermeulen qui a joué un rôle clé dans la conception, la planification et l'exécution du projet. Son ingénuité est même allée jusqu'à concevoir le logogramme du symposium qui apparaît en page-couverture du présent numéro. Nous désirons également exprimer notre sincère reconnaissance à la rédactrice spéciale, Natalie Sutterlin, qui s'est acquittée de l'énorme tâche de trouver des arbitres pour les articles et de préparer les manuscrits pour l'impression.

C'est un privilège pour le *Journal* que de publier ce numéro spécial qui contribuera à augmenter nos connaissances sur les effets de la pollution par le pétrole des côtes de l'océan mondial.

J. C. STEVENSON
*Directeur de la publication*
*et de l'information scientifique*

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## IN MEMORIAM

# Dr Max Blumer
# Organic Geochemist and Environmental Scientist, 1923–77

*In Memoriam Address at the*
*Symposium on "Recovery Potential of Oiled Marine Northern Environments" held at Halifax, Nova Scotia, October 10–14, 1977*

In memoriam addresses of this type are usually presented by colleagues who have been contemporaries for most of a person's scientific career. I am honored and grateful that as a younger colleague of Dr Blumer's I have the opportunity to present this address. Max Blumer's pioneering research in marine organic geochemistry catalyzed much of the research in this field and provided for exciting and rewarding research opportunities for scientists of my generation and for the future. Thus it is appropriate that one of us has the opportunity to review and comment on his distinguished career.

Most of the participants in this conference are familiar with Max's contributions to oil pollution research. These important contributions represent only a portion of his scientific career. Max Blumer was born August 3, 1923 in Basel, Switzerland. During 1943 to 1949 he studied at the University of Basel minoring in physics, physical chemistry, and geology, and majoring in chemistry. His thesis research *Trace Analysis of Sedimentary Rocks and Their Geochemistry* was completed in 1949 and he received a Ph.D. (summa cum laude) from the University of Basel. The year following his graduation, Max taught analytical chemistry at the University of Basel. Two years of post-doctoral fellowship studies in the United States followed: 1950–51 at the

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University of Minnesota conducting research in polarography and 1951–52 at Scripps Institution of Oceanography conducting research on organic matter in sediments.

In 1952 through mid-1953, Max began a period of research on crude oil composition and crude oil geochemistry at BPM-Shell, Amsterdam, and continued this effort between 1953 and 1958 at Shell Development Company, Houston, Texas. From 1958 to 1959 he returned to Basel to work for CIBA Limited on electrochemistry in aqueous and nonaqueous systems and preparative inorganic chemistry. During this period he conducted research which led to two patent applications by CIBA, Ltd., on the production of pure niobium and tantalum and the protection of the surface of niobium and tantalum.

In 1959 Max Blumer came to Woods Hole Oceanographic Institution as an organic geochemist and in 1964 he was appointed as senior scientist.

During his lifetime Max Blumer published over 75 papers and chapters, beginning in 1948 with "Eine Feldmethode zur Wasseruntersuchung mit Ionenaustauschern" in *Experientia*, 4: 351, and in 1950 with his thesis research. These papers in 1950 were the first in a continuing series on porphyrin geochemistry. His contributions included innovative applications of separation chemistry and probe mass spectrometry to the identification of porphyrin dimers and polymeric porphyrins in sediments. Throughout his career Max would periodically return to research on porphyrins and chlorins in sediments. During the brief time I knew Max, 1971–77, he seemed to me to be the most excited and contented when he was extracting, isolating, purifying, and analyzing pigments and their diagenetic products; unraveling their geochemistry; learning more of the carbon cycle in modern and ancient sediments; and understanding more about the origin of fossil fuels.

Max's invitation to come to Woods Hole Oceanographic Institution was planned as the start of a research program concerned with organic compounds in contemporary marine environments. He was one of the leading pioneers in the modification of modern organic chemical analyses for application to marine organic geochemistry. He persuaded the United States Navy to purchase a CEC-104 mass spectrometer for his research effort in the mid-1960s and he personally modified the mass spectrometer and interfaced it with a gas chromatograph, one of the first such research tools available for marine organic geochemistry research.

His applications of microhydrogenation and ozonolysis techniques in concert with gas chromatographic–mass spectrometric techniques to elucidate structures of hydrocarbons and fatty acids in marine samples are acclaimed as elegant. His work on identification of hydrocarbons from marine organisms is one of the main foundations, if not the main foundation, of our present knowledge of biological sources of hydrocarbons in the marine environment.

Throughout this period of research on biosynthesized hydrocarbons, Max maintained an active interest in other aspects of organic geochemistry, including, as previously mentioned, his research on pigments. In 1967 he published a paper on "Equilibria and Non-equilibria in Organic Geochemistry" in an ACS monograph, Advances in Chemistry, No. 67, *Equilibrium Concepts in Natural Water Systems*, American Chemical Society (1967). In this paper he set forth his thinking on the applications of thermodynamics to organic geochemistry. Max repeatedly used variations on the theme of this paper as an example for students and postdoctoral scientists that it is necessary to understand thoroughly the basic chemical principles if we were ever to understand organic geochemistry. He was very concerned that the haste of many young

scientists toward "relevant" environmental research was resulting in inadequate formal education and inexperience in fundamental research principles.

Max provided collaborative research opportunities at Woods Hole Oceanographic Institution for many visiting scientists, summer student fellows, and postdoctoral scientists. Even though Max was a senior scientist, a position in Max Blumer's laboratory did not entail an extensive waiting period before discussing your research problem in some office far removed from the wet-chemistry laboratory. Max's office was a few steps from his own wet-chemistry bench. He taught research by example. He was constantly in the laboratory extracting samples, isolating compounds, repairing gas chromatographs, preparing gas chromatograph columns, operating the mass spectrometer. He was a dynamo of research activity.

In September 1969 the oil barge *Florida* spilled No. 2 fuel oil into Buzzards Bay, Massachusetts, mainly around the shoreline of West Falmouth. Oil came ashore in productive marsh and shellfish areas of West Falmouth. Max Blumer, along with Dr Howard Sanders and Dr John Teal of the Biology Department, and numerous co-workers, responded to requests for assistance from Mr George Souza, Town of Falmouth Shellfish Warden. Because of his research on biosynthesized hydrocarbons in marine ecosystems, Max Blumer's laboratory was well equipped for analyzing samples to measure No. 2 fuel oil components. The task was a difficult undertaking because it meant that the research effort in his laboratory had to be reoriented.

The results of Max Blumer's analyses of the West Falmouth oil spill samples are well known to most of the participants of this conference. It was a pioneering effort! The first application of sensitive and discriminating analytical chemistry techniques to oil pollution problems. Max used fully his knowledge of organic geochemical processes in the marine environment and his knowledge of petroleum chemistry and geochemistry to interpret the results of the analyses. More importantly, Max Blumer made his results known to the public and presented them in a manner which they could understand.

He suggested many areas of real and potential ecological damage from oil pollution and was principal formulator of the basic set of questions about inputs, fates, and effects of oil in the aquatic environment which provided the stimulus for much of the oil pollution research conducted throughout the world.

As much as I would like to, I cannot avoid commenting that as a result of his public discussions of oil pollution problems he was often criticized by individuals from the petroleum industry. Despite direct and subtle pressures to tone-down his pronouncements of oil pollution problems, Max continued to press for oil pollution research and control of oil discharges to the marine environment. When I once asked him what was the use of us beating our heads against a stone wall, he passionately replied that we were funded by the general public to conduct research and when we obtained results which they should know about, it was our obligation to make our results and interpretations known to them.

In 1974, when it was clear that his main message about oil pollution problems had been made known to the scientific community, government officials, the petroleum industry, and the general public, Max turned to other research. He began to investigate the geochemistry of aromatic hydrocarbons in modern and ancient sediments. The results of these investigations, published in the last few years, have set forth dramatically the complexity of biologically active trace organic compounds. He pointed out in his paper on "Organic Compounds in Nature: Limits of Our Knowledge", *Angewandte Chemie*, 14:

507–514, that there is a need for "transition to a more realistic study of nature that acknowledges the limitations of our present analytical powers and the gaps in our understanding."

Max Blumer was a scientist dedicated to excellence in basic research. Max was a leading pioneer in marine organic geochemistry and marine environmental quality research. His timely application of basic marine organic geochemistry research experience to oil pollution problems is an outstanding example of the contributions of basic research to socially relevant problems.

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# Introduction to the Symposium on Recovery Potential of Oiled Marine Northern Environments

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WITH current growing concern over the presence of foreign chemicals entering our living environment (e.g. Addison 1977; Giam et al. 1978) there has been increased interest in the fate and effects of petroleum hydrocarbons, whether from oil spills or from other land-based industrial or domestic sources. Each year sees a number of conferences organized around this concern, most notably the biennial American Petroleum Institute-sponsored meeting on Prevention And Control Of Oil Spills, and the American Institute of Biological Sciences-sponsored meetings emphasizing the biological and ecological aspects. These conferences and others like them are essential in bringing together the various workers with diverse interests in this rapidly growing field, and do much to stimulate further research and exchange of ideas. However, they can not always address those questions of general shared interest that emerge as central themes.

One such central question has been the problem of environmental recovery following an oil spill. As several studies have shown (e.g. Baker 1976; Sanders et al. 1977; Thomas 1977; Vandermeulen and Gordon 1976) recovery following a spill can take several years, but both the pattern of recovery as well as the process of recovery are not understood. It was for this reason that in the fall of 1976 we began to organize this symposium on the recovery potential of oiled northern marine environments, our rationale being that without some idea of the ability of an environment or ecosystem to respond to or absorb a spill or chronic influx of oil any discussion of environmental impact is largely academic. By bringing together the top workers in this field, representing the various disciplines, an integrated discussion of the more general property of environmental recovery processes would hopefully lead to recognition of some general principles common to the various spills studied. The least such a discussion could lead to would be an evaluation of a possible time scale for the total recovery of a polluted beach or bay. The approach seemed reasonable, for over the last few years a number of well-known oil spills have been studied to the point where a sizable body of knowledge and of understanding had become available, and could be brought together under one roof, for comparison and discussion. It was this comparative aspect of the symposium that was very important, for by discussing all known spill studies together it might be possible to extract some common principles of environmental impact and recovery. With the recovery potential of oiled environments as the central theme, and with the 1969 West Falmouth spill and the 1970 *Arrow* spills as focal points, over 160 participants from nine countries met in Halifax, Nova Scotia, to discuss just how much oil an environment can tolerate, what the recovery pattern or patterns may be, and what the weak links or fragile components are in the sequence of recovery steps following a spill.

The format of the meeting consisted of three consecutive sessions, each of about a dozen papers, and each focusing on a different aspect of the main theme of the symposium. Papers were arranged so as to lead naturally into one another, with each session building up on the preceding session. The session chairmen, all recognized experts in their fields, summarized the presented papers both in light of their own understanding of the problem as well as in terms of the main points from the preceding session, and relating the main message of the papers to the main theme of the symposium. In this way the 3½-day meeting built up continuously to a final summary discussion at the end of the last session and the last paper.

Taken in order, the first papers dealt with the chemical and physical changes in the oil following a spill. The presentations then moved to the various absorption

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and degradative mechanisms operating in the environment, be they physical, microbial, or physiological. The final series of papers focused on the long-term recovery patterns, and was built around long-term studies of the *Torrey Canyon* disaster, the West Falmouth incident, the *Arrow*, the *General M. C. Meigs* grounding on the west coast of Washington, and the recent *Metula* accident in the Strait of Magellan. Throughout the symposium the emphasis was on long-term follow-up studies, with strong emphasis on documented field work as distinct from simulated laboratory studies. Some laboratory studies were included so as to illustrate a particular spill phenomenon or to examine certain physiological effects that can be studied in the field only with great difficulty.

What was achieved? It is too simplistic to expect that hard numbers for such a complicated problem would emerge at the end of the symposium. Each spill and each oiled area differs in so many respects that even a simple estimate at recovery time becomes at best an educated guess. That was not the prime purpose. The symposium was held to bring together under one roof the top workers in this field, and to provide an opportunity to examine different spill studies, be they biologically, chemically, or ecologically oriented, in terms of the long-term recovery from a major oil spill. In this the symposium succeeded. The mood during the 2nd and 3rd day grew more thoughtful as the topics changed from the mechanics of oil transport to bioavailability and in turn to the physiological repercussions and enzymatic degradation mechanisms. Figuring very strongly in this was the series of papers in Session III, dealing with long-term ecological recovery patterns. Introduced by Southward and Southward's study of intertidal fauna and floral alteration patterns following the *Torrey Canyon* disaster, 1967 to the present, this last session brought together all the long-term studies available to date. In retrospect this session was almost a research exercise in itself, and probably sufficient reason for having participated in this symposium.

Nonetheless there were a number of points raised that deserve separate mention and comment. Probably the most critical observation made was that raised by Clark and Mann in their summary after the final session, in which they pointed out the oft forgotten observation that natural inherent variations in the environment are part and parcel of nature, and that these variations can often be of a significant magnitude. Thus where a change in tissue growth or in population abundance seems obviously related to some environmental disaster such as an oil spill, these changes may equally well be part of a natural variability in organism growth or abundance, totally unrelated to the oil spill in question. The paper by Dr Notini (Sweden) describing ecological changes relating to a Baltic oil spill illustrates this point. In this study changes in population abundance were found to coincide not only with oiling, but also with fluctuations in surface water temperature. Therefore extreme care must be exercised in the experimental design of these sort of ecological impact studies, and in attributing changes to oil spills and oil exposure. The science of oil spill impact assessment must be as "tight" a science as that of pure research.

Despite this criticism there is, nonetheless, the demonstrated fact of the devastating effect of oil on marine environments, the persistence of petroleum hydrocarbons and the persistence of their biological impact. And this was probably the real message of the symposium. For even though the third session demonstrated the eventual recovery of oiled marine populations and ecosystems, somewhere between 5 and 15 years depending on the spill conditions, papers in Sessions I and II documented repeatedly the persistence of oil, particularly of the larger molecular weight components, for possibly longer duration than the observed ecological recovery times. A number of papers addressed this phenomenon, as well as documenting the changing composition of oil. One common feature of environmentally altered petroleum is the loss of the *n*-alkanes and the lower molecular weight hydrocarbons, with the parallel enhancement of the aromatic components. In fact, Cretney et al. suggest that this long-term resistance to degradation of the large molecular weight residuals, such as the pentacyclic triterpanes, makes these compounds useful candidates for long-term source identification of oil and of oil spills.

Of greater concern is this same persistence of hydrocarbons in tissues, after brief and after chronic exposures of organisms to various oils or oil components.

Organisms do rid themselves of foreign molecules and tissues do depurate, but the mechanism of depuration is not understood, nor is depuration complete in all cases. Loss of tissue-bound hydrocarbons seems to be aided by the cytochrome-$P_{450}$ based aryl hydrocarbon hydroxylase system. At least, this is the case in vertebrate tissues. The situation is less clear-cut in the marine invertebrates where this enzyme system has been identified in a number of phyla, but seems nonexistent in such ecologically and commercially important groups as the bivalve molluscs. The potential implications of (a) a degradative bottle-neck in the marine foodchain, and of (b) bioaccumulation and concentration of the larger aromatic molecular weight compounds in marine tissues seem obvious, albeit very poorly understood. For the fact is that in terms of understanding the potential disruption of marine foodchain kinetics by petroleum hydrocarbons, science is presently only scratching the surface.

Any discussion of the potential of an ecosystem to absorb an oil spill must necessarily include the role of hydrocarbon utilizing bacteria, for microbial degradation is generally thought to be the final long-term degradative step in removal of hydrocarbons from the environment. This applies especially to those hydrocarbons bound up eventually in the sediments. A number of papers, reporting from various spill studies including the *Arrow* and the *Metula* spills, considered this aspect of environmental defense against pollution. It would appear, however, that microbial degradation is less effective than formerly thought, with microbial processes occurring at low rates and being dependent on nutrients other than hydrocarbons. Also, the mechanisms involved in this supposedly ultimate line of environmental defense in hydrocarbon degradation are generally unknown (Stewart and Marks). Perhaps the most startling observation, and of immediate relevance to the northern and arctic environment, was that microbial breakdown of oil trapped within or under arctic ice is almost negligible. Oil trapped in this manner did not show any appreciable changes during the course of a winter (Atlas et al.). The repercussions of these observations, in the field, in terms of northern exploration and development are foreboding at best. More amazing, however, is that these observations have come to light only now, in 1977, 10 years after the *Torrey Canyon* disaster. This indicates a very large gap in our understanding of the potential impact of oil on our environment.

Perhaps the most remarkable point made during the entire symposium, however, did not come from the speaker's platform but arose from the floor during a general discussion, and related to the use of "control" or "reference" sites. For despite all efforts, true hydrocarbon-free reference sites apparently no longer exist. This is not a new observation. Most workers in this field are aware of this dilemma and have attempted to deal with the problem. All reference sites contain petroleum-derived hydrocarbons, and other nonindigenous compounds for that matter, to greater or lesser degree. It should be noted that in many cases this contamination of reference sites is not man-made. Atmospheric transport of forest-fire debris can account for some of this general background contamination. More serious is the fact that a large part is due to man's efforts. Most oceanic shorelines receive a low-level but continuous input of water-borne petroleum hydrocarbons, and these must eventually become available for biological accumulation and metabolic conversion. The same holds true for benthic sediments where concentration in anoxic layers can be high but rates of microbial degradation are very low. Man can and does pollute his environment consistently and very pervasively, a legacy for future generations.

To return to the main theme of the symposium — "what is the recovery potential of oiled marine environments" — the answer is that oiled marine environments will eventually return to a stable state, in from 5 to 15 years. Nature has a battery of defense mechanisms, including self-cleaning by wave action and beach erosion (Owens), biological conversion (Stegeman; Vandermeulen and Penrose; Gordon et al.), and microbial degradation (Atlas et al.; Colwell et al.; Stewart and Marks). However, very little is known of the process of contamination, the manner in which a foreign compound or compounds interfere with the normal functioning of living systems. Petroleum hydrocarbons are, after all, compounds foreign to biological systems, and most are not compatible with the processes of life.

Ultimately of course the question is whether we find the idea of long-term

recovery acceptable. In terms of such other man-made perturbations as the deserts of the Middle East or the denudation of the Scottish Highlands, recovery time of oiled environments seems a small matter (Mann and Clark). But although these examples involved enormous human costs over a much greater time period, there were no changes in the basic processes of life. Those incidents did not leave residues of problem compounds in our environment, and did not have the potential of physiological disruption or of long-term generation of mutagenic or carcinogenic derivatives. We are relieved to learn that environments do recover following oiling. However, is the damage or injury done during the recovery process worth funding more research and spending more money to prevent oil spills or for that matter other chemical contamination? The matter of research funding was raised repeatedly during the course of the symposium. For although a significant amount of money has been directed to petroleum pollution research, both by government and industry, it became apparent that there exists a lack of consistent funding of such essential long-term studies as were the subject of this meeting. Thus several of the studies included in this volume were funded by "bootlegging" funds from other projects to maintain minimal research effort and quality. The decision of course can not be made until all the facts are in, and judging by this symposium of the top scientists in this field, the facts are not in and we are indeed only scratching the surface.

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**Symposium on Recovery Potential of Oiled Marine Northern Environments**

*SESSION I*

## Long-Term Fate of Petroleum Hydrocarbons After Spills — Compositional Changes and Microbial Degradation

CHAIRMEN: J. N. BUTLER AND E. M. LEVY

# Analyses of Aromatic Hydrocarbons in Intertidal Sediments Resulting from Two Spills of No. 2 Fuel Oil in Buzzards Bay, Massachusetts[1]

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We have analyzed the two- and three-ring aromatic hydrocarbons from the Wild Harbor oil spill in September 1969 and the Winsor Cove oil spill in October 1974, in intertidal marsh sediments, using glass capillary gas-chromatographic and mass-fragmentographic analyses. Naphthalenes with 0–3 alkyl substitutions and phenanthrenes with 0–2 substitutions decreased in concentration with time in surface sediments. The more substituted aromatics decreased relatively less and in some cases actually increased in absolute concentration. The changes in composition of the aromatic fraction have potential consequences for the ecosystem and provide insight into geochemical processes of oil weathering.

*Key words:* oil pollution, aromatic hydrocarbons; gas chromatography; gas chromatography–mass spectrometry; geochemistry; marsh; sediments; oil spills

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Utilisant la chromatographie en phase gazeuse sur capillaire de verre et des analyses fragmentographiques de masse, nous avons analysé les hydrocarbures aromatiques à deux et trois noyaux présents dans les sédiments de marais intertidaux et provenant du déversement de pétrole de Wild Harbor en septembre 1969 et de celui de Winsor Cove en octobre 1974. La concentration des naphtalènes avec 0 à 3 substitutions alkyles et des phénanthrènes avec 0 à 2 substitutions diminue avec le temps dans les sédiments de surface. Les aromatiques les plus substitués diminuent relativement moins et même, dans certains cas, augmentent de concentration absolue. Les changements dans la composition de la fraction aromatique peuvent affecter l'écosystème et nous aident à comprendre les processus géochimiques de l'altération du pétrole à l'air.

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THOUGH there have been a number of studies of oil spills incorporated into coastal sediments (Blumer and Sass, 1972c; Burns and Teal 1971; NAS 1975; Mayo et al. 1978; Keizer et al. 1978; among others), there are not much data as yet on the fate of the aromatic fractions of these oils in the environment. Previous studies have focused on the fate of alkanes and cycloalkanes from the spilled oil (Blumer et al. 1970; Blumer and Sass 1972a, b; Burns and Teal 1971). Yet aromatic hydrocarbons are major constituents of oil and generally considered to be the petroleum components that are the most damaging to organisms (Anderson 1977; Rice et al. 1977; NAS 1975; Sanborn and Malins 1977; Roesijadi et al. 1978). If these compounds have a long residence time in surface sediments they might be released from sediments to organisms living in or on the sediments as well as in the overlying water column.

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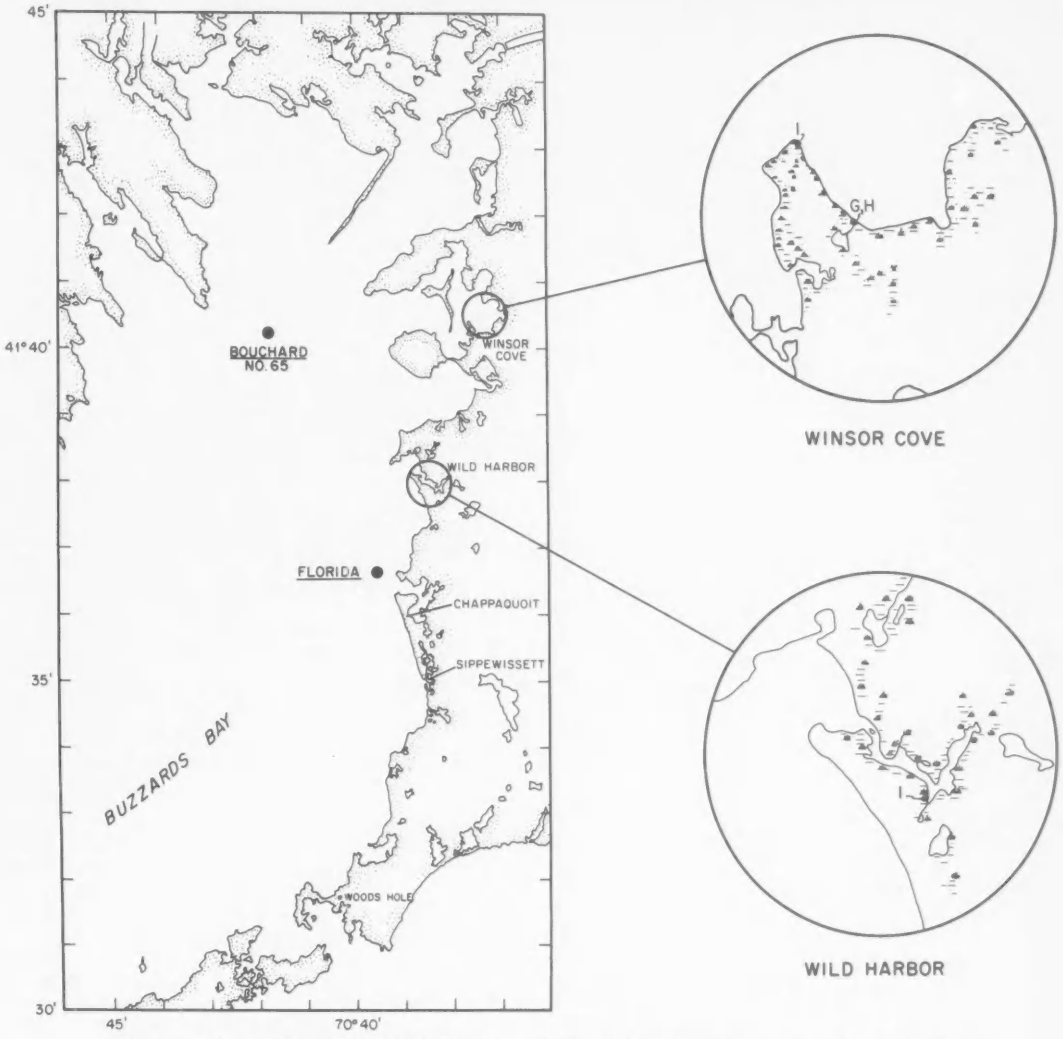


FIG. 1. Chart of locations of Wild Harbor and Winsor Cove in Buzzards Bay, Mass., showing sampling sites.

Thus, it is important to investigate the long-term fate of fossil-fuel aromatic hydrocarbons in surface sediments.

This study is a part of our ongoing efforts to understand the biogeochemistry of fossil fuel compounds in benthic ecosystems (Teal and Farrington 1977; Farrington and Tripp 1975; Farrington et al. 1977; Teal 1976) and part of an overall effort to investigate the fate and effects of a No. 2 fuel oil spill in Buzzards Bay — the West Falmouth oil spill of 1969 (Blumer et al. 1970; Blumer and Sass 1972a, b, c; Burns and Teal 1971; Krebs and Burns 1977; Burns 1976a, b; Sanders 1978) — and a more recent No. 2 fuel oil spill in Buzzards Bay in 1974 (Hampson and Moul 1978) (Fig. 1). We report here a study of the fate of some No. 2 fuel oil aromatic compounds in marsh sediments.

## Materials and Methods

The cores from Wild Harbor were taken at a site located at about midtide that would normally have been covered by *Spartina alterniflora*. This marsh grass was killed by the spill but by the end of the study had recovered to some considerable extent. Below the sample site at lower tidal levels there was soft mud; above the sample site the marsh was continuous to the upland. Sediment at the sampling site was a typical marsh–mud–peat mixture bound together by grass

TABLE 1. Weights of aromatic fractions from marsh sediments — West Falmouth oil spill, September 1969, Wild Harbor station and *Bouchard* Barge No. 65 oil spill, October 1974, Winsor Cove stations.

| West Falmouth spill Wild Harbor station | | *Bouchard* Barge No. 65 spill Winsor Cove stations | |
|---|---|---|---|
| Sampling date | mg/g dry wt[a] | Sampling date | mg/g dry wt |
| Jan. 1971 | | *Site 1* | |
| 0–7 cm | 1.21 | Oct. 1974 | |
| Nov. 1972 | | 0–6 cm (1) | 20.6 |
| 0–7 cm | 2.14 | 0–6 cm (2) | 11.4 |
| Nov. 1973 | | 14–18 cm (1) | 0.12 |
| 0–7 cm | 3.28 | 14–18 cm (2) | 0.24 |
| Sept. 1975 | | May 1975 | |
| 0–7 cm | 1.09 | 0–5 cm | 4.6 |
| July 1976 | | 15–20 cm | 0.05 |
| 0–7 cm | 1.63 | May 1976 | |
| *Control Site Chappaquoit Marsh* | | 0–6 cm | 5.1 |
| | | 25–29 cm | 0.06 |
| | | 45–49 cm | 0.06 |
| May 1975 | | June 1977 | 0.32 |
| 0–6 cm | 0.04 | *Site GH* | |
| July 1977 | | May 1975 | |
| 0–6 cm | 0.02 | 0–6 cm | 0.37 |
| | | July 1977 | |
| | | 0–6 cm (1) | 3.04 |
| | | 0–6 cm (2) | 1.31 |

[a]Gravimetric determination in milligrams per gram dry weight of sediment.

roots. This site was labeled Site 1 in the study of Krebs and Burns (1977) on the response of fiddler crabs to the Wild Harbor oil spill.

Winsor Cove samples came from similar sediments. Site 1 was somewhat higher in elevation, but still was characteristically occupied by *S. alterniflora*. Site GH was at about the same tidal level as the Wild Harbor sample. Control cores were taken from Chappaquoit and Sippewissett marshes several kilometres south of the oiled marshes and similar in tidal levels.

Cores of marsh sediments or intertidal mud-flat sediments were obtained with a stainless-steel coring device of 4 cm diameter and 120 cm length or by inserting plastic core-liner tubes into the marsh sediments. In the latter case care was taken to remove the outside ring of sediment which was in contact with the liner to avoid contamination. The cores were sectioned and frozen at a temperature below 0°C until analysis 1 wk–1 yr later. Analysis of a section of core stored for 2 yr did not give significantly different results from analysis of another section immediately after collection. The methods of extraction and isolation of the hydrocarbons have been described in detail previously (Farrington and Tripp 1975; Burns and Teal 1971; Farrington et al. 1976; Blumer and Sass 1972a). Briefly, the sediments were extracted by Soxhlet extraction with methanol or benzene: methanol (1:1) or by alkaline (KOH) methanol:benzene digestion. The hydrocarbons plus some of the other lipids were partitioned into pentane, concentrated by rotary evaporation, and then percolated through a copper column to remove sulfur. Two hydrocarbon fractions were collected by column chromatography through alumina (5% water) over silica (5% water). Fraction 1 was eluted with one column volume of pentane and contained primarily alkanes and cycloalkanes. Fraction 2 was eluted by one column volume of 10% benzene in pentane followed by one column volume of 20% benzene in pentane and contained primarily two- and three-ring aromatic compounds. Column eluates were concentrated by rotary evaporation at or below room temperature and aliquots were weighed on a Cahn electrobalance.

The aromatic compounds in fraction 2 were analyzed by gas chromatography on a 0.32 mm ID × 20 m glass capillary SE 52 column installed in a Hewlett Packard Model 5710A gas chromatograph (GC) equipped with a flame ionization detector and Model 18740A capillary column control split/splitless injector. Samples were injected using the splitless mode with the column at 40°C and held at that temperature until the solvent peak eluted. The column was then rapidly heated to 70°C and immediately programmed at 2°C/min to 240°C. Gas flow rate was 2–3 mL/min helium. Detector temperature was 250°C and injection port temperature was 200°C.

Quantitation was achieved by comparison of peak heights of compounds in samples with peak heights of an internal standard, hexamethyl benzene, or by comparison with an external standard calibration plot of several aromatic hydrocarbon standards.

The aromatic compounds were also analyzed by GC–mass spectrometry (MS) on a Finnigan 1015 quadruple mass spectrometer coupled to a Varian 1400 gas chromatograph equipped with a split/splitless injection. A 17 m × 0.32 mm ID SE 52 glass capillary column was installed in the system and operated as follows. Injection was at 40°C with 30 sec in splitless mode, then rapidly brought to 70°C and programmed to 240°C at 4°C/min. Helium carrier gas was at 14 psi. The mass spectrometry operating conditions were 20 eV, 500 μA, scan from 40 to 500 u every 3 s. Mass-fragmentographic quantitative analyses were obtained by the general procedure outlined in Farrington et al. (1977) except that the precision was only ±20% due to the small number of scans per peak as a result of the long scan time. Mass spectra for each peak selected for analysis were compared where possible with the API reference spectra (API 1977) and compounds identified in this manner. In many cases reference spectra were not available and compounds were tentatively identified by comparison of their spectra with known spectra for isomers or compounds with similar structure characteristics.

## Results

### GRAVIMETRIC DATA

The concentrations of the $f_2$ aromatic fractions in marsh sediments are given in Table 1. Note that the error associated with analysis of subsamples of marsh sediments from one location are given by the duplicates of three of the Winsor Cove samples. Variation of two- or three-fold is not uncommon due to the heterogeneity of the marsh sediment and the nonuniform distribution of the oil in these heterogeneous sediments. In five Wild Harbor samples we calculated weights of extracted hydrocarbons from the GC's and found they accounted

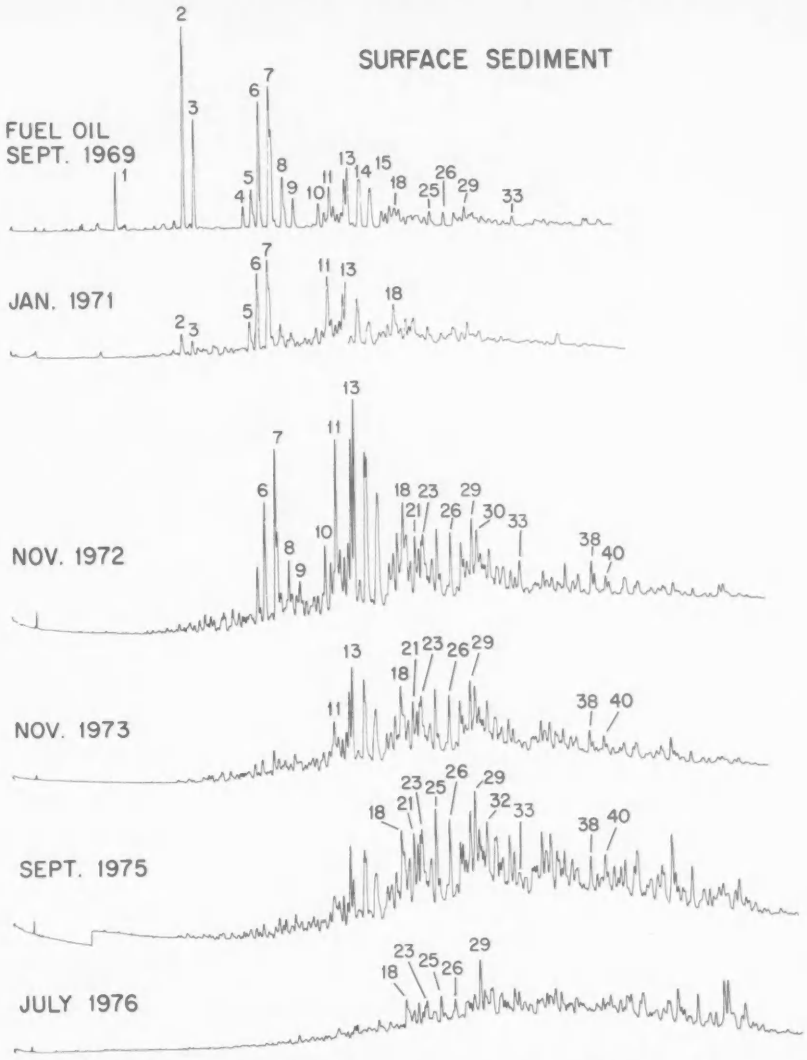


FIG. 2. Gas chromatograms of aromatics from marsh surface sediments, Wild Harbor. (SE-52 gas capillary column.) See Table 2 for identity of numbered peaks. The top trace shows the oil itself, not a field sample.

for 87 ± 7% (SE) of the actual weights. The concentrations of the $f_2$ aromatics in the Wild Harbor marsh sediments remained fairly constant over the time interval sampled. Unfortunately, we did not sample during the first 1.5 yr after the spill and thus cannot directly compare the results with the first samples of the Winsor Cove marsh sediments which were taken shortly after the spill in that location.

At Site 1 in the Winsor Cove marsh the concentrations of the $f_2$ aromatics decreased by a factor of 5 between the October sampling just 3 d after the spill and the May 1975 sample taken about 6 mo after the spill. A year later the concentrations at Site 1 were the same as the May 1975 data. However, by June of 1977 the concentration had decreased again by a factor of 10. Curiously, the concentrations at Site GH increased by an order of magnitude during this same period. We cannot explain this except to hypothesize that Site GH is near a small flowing stream and might have received some addition to the aromatic fraction from sediments "upstream" or farther up the tide zone.

The concentrations of $f_2$ aromatics normally present

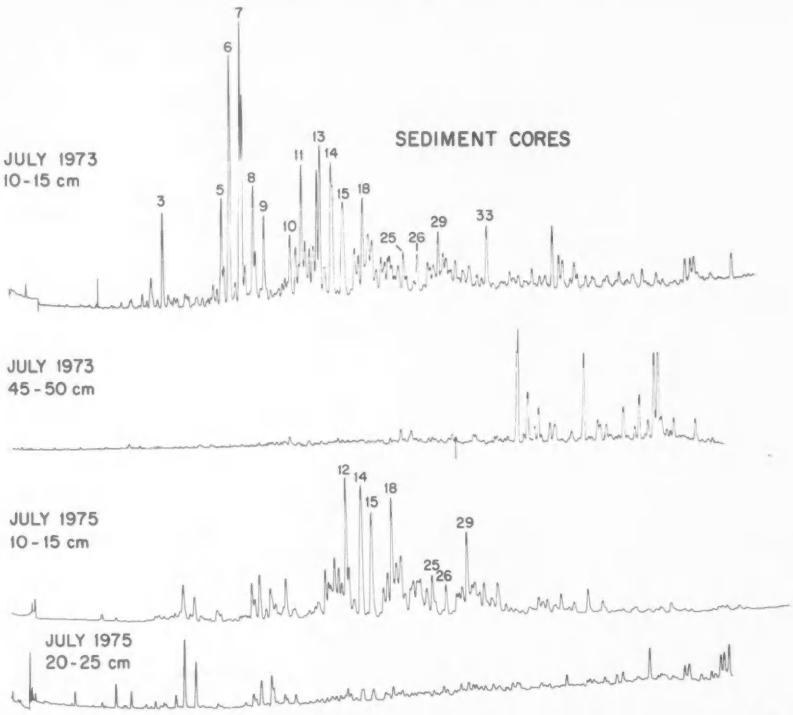


FIG. 3. Gas chromatograms of aromatic compounds from marsh core sections, Wild Harbor. See Fig. 2 for explanation.

in the marsh sediments are given by the values for the control marsh samples at Chappaquoit and the lower sections of the marsh cores for Site 1 in Winsor Cove. The analytical blank for these analyses is about 1 order of magnitude lower in concentration than the control station values.

## GLASS CAPILLARY GAS CHROMATOGRAPHY

Glass capillary gas chromatograms of representative samples are given in Fig. 2, 3, and 4. For all figures of gas chromatograms the identity of numbered components as near as can be determined by GC–MS data and retention indices of standard compounds are given in Table 2. Inspection of the chromatograms in Fig. 2 clearly shows that between the initial spill at Wild Harbor in September 1969 and January 1971 there was a removal of lighter molecular weight components at a faster rate than removal of the heavier molecular weight components. This decrease of lighter molecular weight components relative to the heavier components continued from January 1971 to July 1976. The dominance of the methylnaphthalenes (compounds 2 and 3) as the most abundant individual aromatics in the aromatic fraction of the fuel oil gives way to a dominance of $C_2$-naphthalenes by January 1971, $C_3$-naphthalenes by November 1972, and $C_4$-naphthalenes by September 1975.

Chromatograms of aromatic compounds present at selected depths in Wild Harbor marsh cores are given in Fig. 3. Note that fuel-oil aromatics are present at 10–15 cm in July 1973 and the methyl- and dimethylnaphthalenes do not appear to be as depleted relative to the heavier molecular weight compounds as, for example, the November 1972 marsh surface sediments shown in Fig. 2. The 45–50-cm core section chromatogram (Fig. 3) shows an absence of fuel-oil aromatics. The identities of the peaks in this chromatogram are not known at present but they are probably aromatic hydrocarbons from pyrolitic sources (Youngblood and Blumer 1975). The chromatograms of the July 1975, 10–15-cm section (Fig. 3) show some fuel-oil aromatics present. The lighter molecular weight components are reduced relative to the heavier molecular weight components when compared with the July 1973 analyses of the 10–15-cm core section from this location. Traces of fuel-oil aromatics are also present in the 20–25-cm section of the July 1975 core. The lighter molecular weight components are present in relatively large amounts so that the chromatogram re-

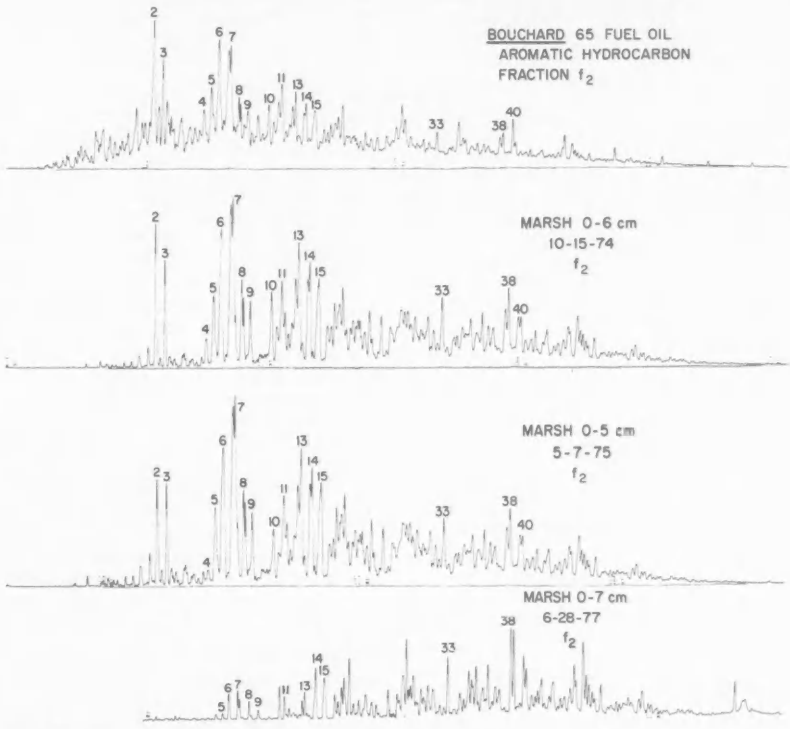


FIG. 4. Gas chromatograms of aromatic compounds from marsh surface sediments, Winsor Cove. See Fig. 2 for explanation. Top trace is of oil that was spilled, second trace is initial spill sample extracted from sediment.

sembles that of the original oil (Fig. 2). The trace amount of the sample prevents strict comparison with the original oil.

Chromatograms of the aromatics in the No. 2 fuel oil from the *Bouchard* Barge No. 65 spill and representative samples of marsh surface sediments are presented in Fig. 4. In this series of samples we have captured the early stages of the processes governing the fate of the spilled oil in the sediments that we missed for the Wild Harbor samples. The sequence of decreasing relative abundance of the lighter molecular weight aromatics occurs over the 2.5 yr sampled. However, the October 1974 sample taken just 3 d after the spill contains a suite of aromatics almost identical in relative abundance with the original oil as determined by our analyses. Between October 1974 and May 1975 there was some depletion of the methylnaphthalenes and $C_2$-naphthalenes relative to heavier molecular weight components. After that, the pattern set by Wild Harbor data is repeated as time progresses.

We have measured the concentrations of several of the classes of aromatics in the Winsor Cove marsh sediments using the GC and GC–MS data set. The total concentration of all the naphthalenes and phenanthrenes measured decreases in concentration over the time interval sampled. The data normalized to $C_3$-naphthalene concentration (Table 3) show that over the first 6 mo all the groups of compounds decreased in concentration at the same rate. However, from May 1975 to June 1977 these compounds had different rates of loss with the lighter molecular weight components decreasing at a faster rate. Concentrations of selected individual aromatic compounds from the Wild Harbor marsh show a similar picture over a longer time period (Table 4). The lighter compounds decrease throughout the 7-yr period in absolute and relative concentration. Intermediate weight compounds give indications of an increase in concentration during the middle of the study period, followed by a decrease, while the heaviest compounds increase relatively and absolutely in concentration throughout the 7 yr.

## GLASS CAPILLARY GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC COMPUTER SYSTEM DATA

Computer-reconstructed total-ion chromatograms from the GC–MS analyses for three of the samples are given in Fig. 5. Computer-reconstructed selected-ion

TABLE 2. Compounds list for Fig. 2–6. GC, gas-chromatography retention indices; MS, mass-spectral interpretation; MSA, mass-spectra match with API reference spectra.

| Peak no. | Compound | Method of identification |
|---|---|---|
| 1 | naphthalene | GC and MSA |
| 2 | 2-methylnaphthalene | GC and MSA |
| 3 | 1-methylnaphthalene | GC and MSA |
| 4–9 | $C_2$-naphthalenes | MSA |
| 10–17 | $C_3$-naphthalenes | MSA |
| 18 | $C_4$-naphthalene | MSA |
| 19 | tetrahydrophenanthrene | MS |
| 20–29 | $C_4$-naphthalenes | MS |
| 30 | $C_4$-naphthalene + $C_1$-fluorene | MS |
| 31 | $C_4$-naphthalene | MS |
| 32 | dibenzothiophene | MSA |
| 33 | phenanthrene | GC and MSA |
| 35 | $C_2$-fluorene | MS |
| 36 | methyldibenzothiophene | MSA |
| 37 | methyldibenzothiophene | MSA |
| 38 | methylphenanthrene | MSA |
| 39 | methylphenanthrene | MS |
| 40 | methylphenanthrene | MS |
| 41 | methylphenanthrene | GC and MS |
| 42–44 | $C_6$-naphthalenes | MS |
| 45–50 | $C_2$-phenanthrenes | MS |
| 51–54 | $C_7$-naphthalenes | MS |
| 55 | $C_3$-phenanthrene | MS |
| 56 | $C_3$-phenanthrene | MS |
| 57–58 | $C_3$-phenanthrenes | MS |

TABLE 3. Concentrations (μg/g dry wt) of naphthalenes and phenanthrenes in Winsor Cove marsh surface sediments (0–6 cm).

| Compounds | Oct. 1974 | May 1975 | June 1977 |
|---|---|---|---|
| naphthalene | 9.2 | 0.63 | 0.11 |
| $C_1$-naphthalenes | 370 | 41 | 1.2 |
| $C_2$-naphthalenes | 1380 | 260 | 15 |
| $C_3$-naphthalenes | 3040 | 590 | 33 |
| $C_1$-phenanthrenes | 500 | 97 | 15 |
| $C_2$-phenanthrenes | 480 | 96 | 27 |
| *Normalized to $C_3$-naphthalenes* | | | |
| $C_1$-naphthalenes | 0.12 | 0.07 | 0.04 |
| $C_2$-naphthalenes | 0.45 | 0.44 | 0.45 |
| $C_3$-naphthalenes | 1.00 | 1.00 | 1.00 |
| $C_1$-phenanthrenes | 0.16 | 0.16 | 0.46 |
| $C_2$-phenanthrenes | 0.16 | 0.16 | 0.82 |

plots of molecular ion mass–charge ratios for phenanthrene, $C_4$-naphthalenes, $C_1$-phenanthrenes, $C_5$-naphthalenes, $C_2$-phenanthrenes, $C_6$-naphthalenes, $C_3$-phenanthrenes, and $C_7$-naphthalenes are given in Fig. 6. Such plots do not necessarily indicate the presence of these compounds. Rather they indicate whether or not these compounds are likely to be present as components of the sample and which mass spectra should be examined closely for evidence of these compounds. We inspected mass spectra of the peaks, which were enhanced in these selected-ion plots compared with total-ion plots, and where possible identified the compound or compounds present where the peak eluted. This approach allowed us to identify several of the compounds and tentatively identify several others. For example, we identified peak number 32 as dibenzothiophene and peaks 36 and 37 as methyldibenzothiophene based on a match of the mass spectra with spectra for those compounds given in the API reference spectra (API 1977).

Unfortunately, we could not find reference spectra for most of the $C_3$–$C_5$ naphthalenes and phenanthrenes nor were commercial standards available to generate reference spectra and gas-chromatographic retention indices. Thus our identifications of several of the compounds in Table 2 are incomplete and tentative based

TABLE 4. Concentrations (μg/g dry wt)[a] of selected aromatic hydrocarbons in Wild Harbor marsh sediments. NM, not measured; NA, not applicable. Peak numbers are in parentheses.

| Compound | Sept. 1969[b] | Jan. 1971 | Nov. 1972 | Nov. 1973 | Sept. 1975 | July 1976 |
|---|---|---|---|---|---|---|
| $C_2$-naphthalene (6) | NA | 23.4 | 8.6 | 3.2 | 0.9 | trace |
| $C_3$-naphthalene (13) | NA | 20.3 | 16.2 | 19.8 | 2.7 | 0.45 |
| $C_4$-naphthalene (25) | NA | 5.4 | 5.9 | 14.9 | 6.8 | 1.4 |
| $C_1$-phenanthrene (38) | NA | NM | 8.6 | 15.8 | 7.2 | 0.9 |
| $C_2$-phenanthrene (47) | NA | NM | 5.0 | 23.0 | 23.0 | 7.2 |
| $C_3$-phenanthrene (57) | NA | NM | 7.4 | 9.2 | 8.2 | 14.4 |
| | *Normalized to $C_3$-naphthalene* | | | | | |
| $C_2$-naphthalene (6) | 1.4 | 1.2 | 0.53 | 0.16 | 0.33 | trace |
| $C_3$-naphthalene (13) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| $C_4$-naphthalene (25) | 0.21 | 0.27 | 0.36 | 0.75 | 2.5 | 3.1 |
| $C_1$-phenanthrene (38) | 0.32 | NM | 0.53 | 0.80 | 2.7 | 2.0 |
| $C_2$-phenanthrene (47) | 0.32 | NM | 0.31 | 1.2 | 8.5 | 16 |
| $C_3$-phenanthrene (57) | 0.06 | NM | 0.46 | 0.46 | 3.0 | 32 |

[a]Total estimated error from analytical measurement and sediment subsample heterogeneity is ±20 to 30%.

[b]Data for spilled oil.

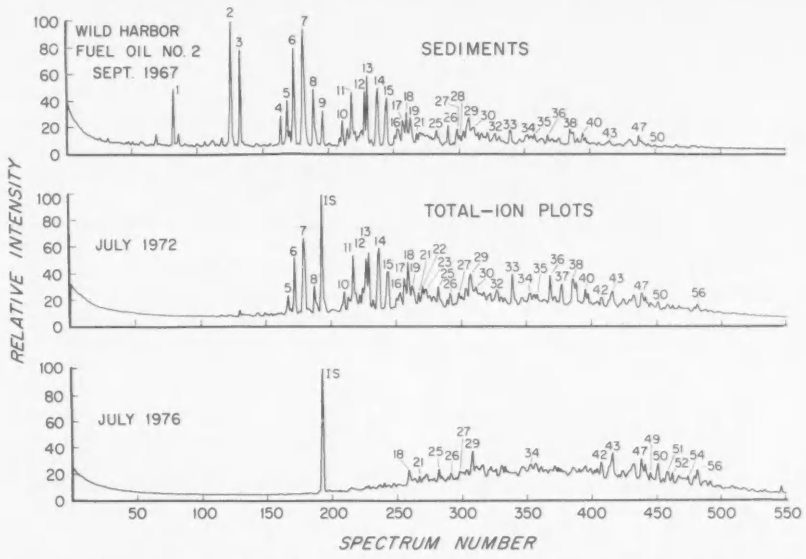


FIG. 5. Computer-reconstructed total-ion plot of GC–MS analyses of aromatic compounds of marsh surface sediments, Wild Harbor. See text for details of analyses conditions.

on interpretation of the mass spectra.

The selected-ion plots in Fig. 6 illustrate the more rapid losses of the $C_4$-naphthalenes, phenanthrene, and methylphenanthrenes relative to the $C_5$- and $C_6$-naphthalenes and the $C_2$-phenanthrenes. Note that the dibenzothiophene (peak 32, Fig. 6) and methyldibenzothiophene (peaks 36, 37 Fig. 6) concentrations decrease more slowly than the adjacent aromatic hydrocarbons, phenanthrenes, and methylphenanthrenes (peaks 33, 38, 39, 40, and 41, respectively) when comparing the data from the original No. 2 fuel oil and the July 1972 sample. Gas-chromatographic–mass-spectral selected-ion plots for the Winsor Cove samples gave the same result.

## Discussion

Two local marshes were contaminated by spilled No. 2 fuel oil at the same time of year, 5 yr apart. Our investigations show that the aromatic compounds present in the spilled oil had similar fates. Lighter molecular weight compounds decreased in concentration more rapidly than heavier molecular weight compounds, e.g. naphthalene, $C_1$- and $C_2$-naphthalenes compared with phenanthrenes. By the end of the study periods, most of the aromatic compounds had decreased in absolute concentration. But the heaviest ones in Wild Harbor marsh were actually present in larger amounts than earlier in the study, although they may have been less concentrated than they were immediately after the spill since we have no data for that period. There is a wide variety of aromatic hydrocarbons present in marsh sediments at concentrations well-above background, 6.5 yr after the spill of No. 2 fuel oil.

The most reasonable explanation for the decreasing concentrations of most of the aromatics is a combination of diffusion, water solubilization or accommodation and transport from the sediments, evaporation, and perhaps microbial attack (Gibson 1977). Water washing and/or evaporation could result in a more rapid removal of the lighter molecular weight compounds as they have greater solubility and higher vapor pressure (McAuliffe 1977; Boehm and Quinn 1973; Eganhouse and Calder 1976). The absolutely higher concentration of the heaviest compounds must be explained by a concentrating of the remaining oil in surface sediment vertically in the peat and horizontally in the study area. Selective absorption–desorption processes would play a role in such concentration. Our samples came from a midtide region where marsh sedimentation is most rapid. As erosive processes are likely to be most active near high and low water (Owens 1978), these particular conditions could lead to just such a concentration.

The uniform decrease of aromatics from October 1974 to May 1975 for the Winsor Cove samples could be explained as follows: The marsh sediments were so saturated with oil, as indicated by the high concentrations of aromatic hydrocarbons (Tables 1 and 3), that water washing removed the bulk oil without partitioning with the sediment. As the concentration of the oil was lowered, sediment–oil–water interactions became more important than the simpler water–oil interactions which first took place. By water–oil interaction we refer to interactions between the oil and the sum of seawater and its constituents including the macromolecular weight humic and fulvic materials which promote accommodations of some hydrocarbons into seawater

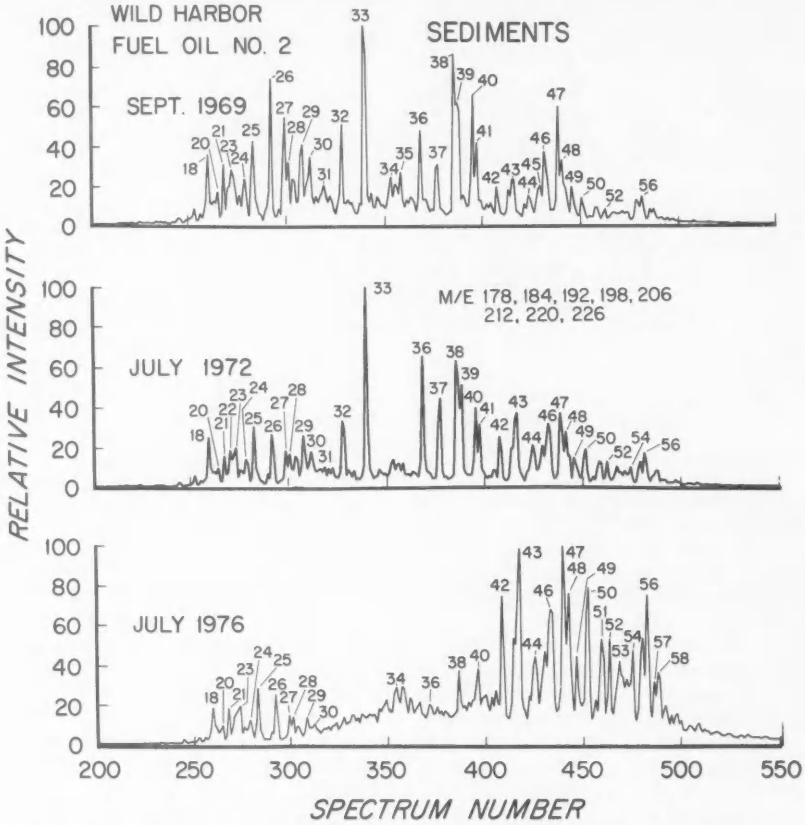


FIG. 6. Computer-reconstructed selected-ion plot of GC–MS analyses of aromatic compounds in marsh surface sediment, Wild Harbor. See text for details of analyses conditions. Some analyses as given in Fig. 5, except for different data plot.

(Boehm and Quinn 1973). The relative importance of the solubilization, evaporation, and microbial degradation of the aromatics cannot be discerned from our data. Oil slicks floating on the surface of the water as the tide came in were observed for several years after the spill in the absence of other disturbance of the mud. This demonstrates that water washing of oil out of the sediments did occur over that time period.

Analyses of a few core sections from the Wild Harbor marsh showed that aromatics were incorporated below the upper 0–7 cm to a depth of 25 cm. The aromatics incorporated in the deeper sections of the marsh core were not as weathered as those in the surface sediments sampled at about the same time.

Most of the experiments or measurements of uptake and depuration of aromatic compounds from petroleum have been conducted with naphthalene and $C_1$- and $C_2$-naphthalenes, although a limited number of experiments have dealt with heavier molecular weight compounds such as phenanthrenes (Anderson 1977; Rice et al. 1977). Results to date suggest that the heavier molecular weight aromatics have a longer residence time than lower molecular weight aromatics once they are incorporated into animal tissue (Roesijadi et al. 1978; Lee 1977). Our measurements show that organisms living in the marsh were exposed to heavier molecular weight fuel-oil aromatics for at least 6.5 yr. The extent to which organisms take up and incorporate these aromatics in their tissues under these conditions of exposure is not known at present. Experiments by Roesijadi et al. (1978) and Gordon et al. (1978) show that organisms living in conjunction with oiled sediment do take up small amounts of oil during short-term exposure of a few weeks. When our data are combined with theirs, it seems clear that investigation of the toxicology, biochemistry, and geochemistry of aromatic compounds over time periods of years will be needed to understand the long-term fate and effects of petroleum compounds in marsh ecosystems, whether they are subjected to acute spills or chronic releases of oils. Clearly the heavier aromatics in No. 2 oils, the highly substituted naphthalenes and phenanthrenes, are the

compounds that could have adverse affects for periods that may well be measured in decades.

Krebs and Burns (1977) in their studies of fidler crabs (*Uca*) in the Wild Harbor marsh found that there was an inverse correlation between the amount of aromatics in the sediments and the size of the crab population. Our data that come from their "Station 1" show that the lighter aromatics are responsible for most of the immediate and toxic effects. They have other stations where recovery has still not occurred but for which we do not as yet have detailed data on the aromatics. We do have mud core data from 1975 that are not from their Station 1 and that show aromatics in relative concentrations much like that of the original oil, although the absolute concentration is very low. However, a small crab overwintering in the sediment would be in potential contact with that oil with its light aromatics for several months and the initial overwintering period is a time of principal mortality associated with oil. But all of the crabs, even those living in the areas where the population has built up again, are in long-term contact with the heavily substituted naphthalenes and the phenanthrenes. Possible effects of these compounds have been, up to the present time, masked by the toxicity of the lighter fractions. We do not know to what extent these compounds are taken up by the animals. Further and more detailed studies are necessary to see whether the physiology and/or population structure will be affected by long exposure to these compounds.

Our data also bear on the question of the origin of aromatic hydrocarbons in marsh and subtidal surface sediments not polluted by oil spills. Measurements by Giger and Blumer (1974), Youngblood and Blumer (1975), Farrington et al. (1977), Hites et al. (1978), LaFlamme and Hites (1978), Grimmer and Boehnke (1975), among others, all indicated the presence of aromatic hydrocarbons from pyrolytic sources in soils and surface sediments in marshes, lakes, and oceanic areas. Several of these investigations reported depth distributions of these compounds that indicated that a historical record of increasing input of these compounds to sediments had occurred over the past 100 yr as a result of increased burning of fossil fuels and release of pyrolytically formed aromatics to the environment. There was a slight but unlikely possibility that some biogeochemical processes acting on chronically spilled oil (Farrington et al. 1977) incorporated into sediments would change the distribution of aromatics found in petroleum (Youngblood and Blumer 1975; LaFlamme and Hites 1978) to the distinctive distribution of aromatics resulting from pyrolysis and that chronic small oil spills accounted for increased concentrations in some locations. This would require an enhancement of the parent aromatics relative to the alkyl-substituted aromatics once the oil was spilled. For example, phenanthrene would have to increase relative to $C_1$- and $C_3$-phenanthrenes. As is readily seen by inspection of the data in Tables 3 and 4 and Fig. 2, 4, 5, and 6 this does not occur in the marsh sediments, after 6.5 yr of biogeochemical weathering. We think that the Wild Harbor data are the longest recorded field test data available that demonstrate that the conversion of aromatic distributions from spilled oils to that of a pyrolytic source distribution does not occur once oil is spilled to the environment, confirming the projections based on physical, chemical, and geochemical principals (Hase and Hites 1976; Farrington et al. 1977; LaFlamme and Hites 1978).

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# Long-Term Fate of a Heavy Fuel Oil in a Spill-Contaminated B.C. Coastal Bay[1]

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The fate of accidently spilled No. 5 fuel oil in a small coastal bay in British Columbia was observed 6 times during 4 yr. The oil's composition was first changed by loss of the lower molecular weight components by evaporation and dissolution. Biodegradation accounted for almost complete removal of *n*-alkanes in the 1st yr. Pristane and phytane were biodegraded more slowly, but were almost completely gone in 4 yr. The non-*n*-alkane components in the $nC_{28-30}$ range seem to be the most resistant to degradation of all the components resolved in the gas chromatograms. The resistance to degradation of these components indicates their potential for long-term studies of oil spills.

*Key words:* petroleum, fate, environment, gas chromatography, *n*-alkanes, pristane, marine fauna and flora

CRETNEY, W. J., C. S. WONG, D. R. GREEN, AND C. A. BAWDEN. 1978. Long-term fate of a heavy fuel oil in a spill-contaminated B.C. coastal bay. J. Fish. Res. Board Can. 35: 521–527.

Nous avons fait des observations à 6 reprises en 4 ans sur le sort du fuel-oil nº5 déversé accidentellement dans une petite baie côtière de la Colombie-Britannique. La composition du pétrole changea d'abord en perdant, par évaporation et dissolution, ses constituants de faible poids moléculaire. La biodégradation est responsable de la disparition presque complète des *n*-alkènes durant la première année. La biodégradation du pristane et du phytane fut plus lente, mais ils avaient presque complètement disparu après 4 ans. Les composants autres que les *n*-alkanes dans la gamme $nC_{28}$ à $nC_{30}$ semblent les plus résistants à la dégradation de tous les composants séparés dans les chromatogrammes en phase gazeuse. A cause de leur résistance à la dégradation, ces composants pourraient servir aux études à long terme de déversement de pétrole.

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ON January 24, 1973, the freighter *Irish Stardust* ran aground between the northern end of Vancouver Island and the mainland of British Columbia on Haddington Reef in Broughton Strait. The accident caused the release of about 200 tons of "1000 second"[2] fuel oil, which was deposited along shores to the east of the reef by the receding high tide on the morning of January 25, 1973. A major cleanup operation was conducted, but the scientific value of studying the natural fate and effects of spilled oil in an intertidal zone was recognized. Accordingly a small isolated bay was left undisturbed for scientific study. This bay was code-named "Reserved Bay" and this report documents the fate of the oil stranded there.

## Materials and Methods

Six visits to Reserved Bay were made and samples were collected in accordance with the schedule shown in Table 1. All samples were stored in tightly capped brown bottles and frozen upon arrival in the laboratory.

Samples were prepared for analysis by gas chromatog-

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[2]Specifications fueling report, Osaka, Japan: sp gr (15°C), 0.9412; viscosity (R.W. No. 1), 465 cP; % carbon, 9.0; F.P., 91°C; P.P., −10°C; % sulfur, 2.41.

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TABLE 1. Samplings and observations in Reserved Bay.

| Visit | Date | Number of samples collected: Floating oil | Landed[a] oil | Sand | Gravel[b] | Rock-face scraping | Rockweed[c] | Photographs (fixed location[e]) | Observers |
|---|---|---|---|---|---|---|---|---|---|
| I | Jan. 30, 1973 | 1 | 3 | 8 | 0 | 2 | 1 | 20 (6) | Chemist |
| II | Mar. 9, 1973 | 0 | 2 | 6 | 4 | 2 | 2 | 20 (6) | Chemist, biologist |
| III | June 5, 1973 | 0 | 2 | 7 | 3 | 2 | 1 | 20 (6) | Chemist, biologist |
| IV | Aug. 28, 1973 | 0 | 3 | 10 | 2 | 2 | 0 | 20 (6) | Chemist, biologist |
| V | Jan. 26, 1974 | 0 | 1 | 4 | 3 | 2 | 1[d] | 20 (6) | Chemist, biologist |
| VI | May 17, 1977 | 0 | 0 | 6 | 6 | 3 | 0 | 20 (6) | Chemist |

[a]Beached oil not associated with any substrate. [b]Stones 2–5 cm diam, 1–5/sample. [c]From top of beach. [d]Newly deposited. [e]Photographs, taken each visit from fixed positions, of the same easily identifiable features.

raphy in basic accordance with the method of Zafiriou et al. (1972). Oil, oil–water mixtures, or heavily oiled sand was placed in a stoppered centrifuge tube, vigorously shaken with carbon disulfide, and centrifuged. The carbon disulfide solution was decanted into a glass vial and sealed with a cap containing a Teflon-laminated silicone septum. Gravel and similarly large substrates were washed with carbon disulfide and the washings centrifuged. If it was practicable, an optimal oil concentration of 0.2 g/mL was prepared.

Sample solutions were analyzed with two columns: 3% Dexsil 300 on 100/200 mesh Chromasorb W (acid washed) in 10 ft (3.05 m) × ⅛ in. (3 mm) OD stainless steel tubing and 12.5% FFAP on 80/100 mesh Chromasorb G (acid washed, DMCS treated) in 10 ft × ⅛ in. OD stainless steel tubing. A flame ionization detector was used and each column was temperature programmed, the former from 70 to 400°C and the latter from 100 to 260°C. In the case of the Dexsil column a Varian Aerograph 1400 gas chromatograph was used with a nitrogen carrier gas at a flow rate of 20 mL/min and temperature program rate of 6°C/min for samples from the first five site visits. For the samples from the sixth visit, the Dexsil column was used in a Hewlett-Packard 5710A gas chromatograph with helium carrier gas at a flow rate of 30 mL/min and a temperature program rate of 8°C/min. The FFAP column was used in a Hewlett-Packard 5710A gas chromatograph with helium carrier gas at a flow rate of 30 mL/min and a temperature program rate of 8°C/min. The position of individual *n*-alkanes in the chromatograms of oil samples were verified by co-injection of an *n*-alkane standard mixture. A baseline chromatogram was run immediately prior to chromatography of each new sample.

## Observations and Results

The north and south sides of Reserved Bay are bound by vertical rock faces 1.5–3.5 m high. Its head is bordered by a lowland of meadow and marsh grasses. A small stream flows onto the south end of the beach. The low-tide zone consists of a clay-based mud flat; the middle-tide zone of stone, sand, and pebble areas; and the high-tide zone of rock faces and patches of sand, stone, and pebbles. The area surrounding the stream is mostly a sand–clay mixture.

Reserved Bay was visited for the first time 5 d after the spill on January 30, 1973. An oily sheen was visible on the surface waters of the bay; oil-soaked material and thick black patches of oil floated near the beach. A band of oil covered a vertical height of approximately 2 m downwards from the high-tide mark. Except for the southern rock faces that were relatively unoiled, coverage was continuous over rocks, rock faces, logs, and seaweed, with a coating of 1–5 mm thick. The sand was oily, but not coated. A thick bed of detached, heavily oiled rockweed (*Fucus distichus*) covered the high-tide area at the head of the bay. Below the band of contamination the beach appeared to be totally oil-free.

Gas chromatograms of oil samples were very similar in appearance. Visual comparison of the chromatograms of floating or stranded oil with the chromatogram (Fig. 1A) of the original oil indicated a slight diminution of the *n*-alkanes, *n*-decane through *n*-pentadecane in the former as the only obvious difference.

On March 9, 1973 Reserved Bay was visited again. The areas of beach and rock visibly contaminated by the oil were unchanged from the first visit. No apparent migration of oil down the beach or to adjacent unoiled areas had taken place. Many dead limpets (*Acmaea spp.*) were found at the base of the heavily oiled rock faces. Many oil-covered amphipods (*Orchestra sp.*) were found and although most were alive, they exhibited slow restrained movements. Periwinkles (*Littorina spp.*) among the oiled rocks were lying free on the ground with their opercula tightly in position.

In general, the oil looked remarkably unchanged from its appearance in the first visit. It did seem, however, to be slightly less glossy and sticky. The gas chromatograms of samples corroborated the field observations. These chromatograms were not noticeably different from those of samples from the first visit except in a further slight truncation of the lower molecular weight *n*-alkanes.

By June 5, 1973 the location of visible oil contamination was still unchanged. Nearly all the limpets were gone from the heavily oiled rock faces, although a few appeared to be successfully grazing on the oiled rocks. A few amphipods were present in oiled and unoiled

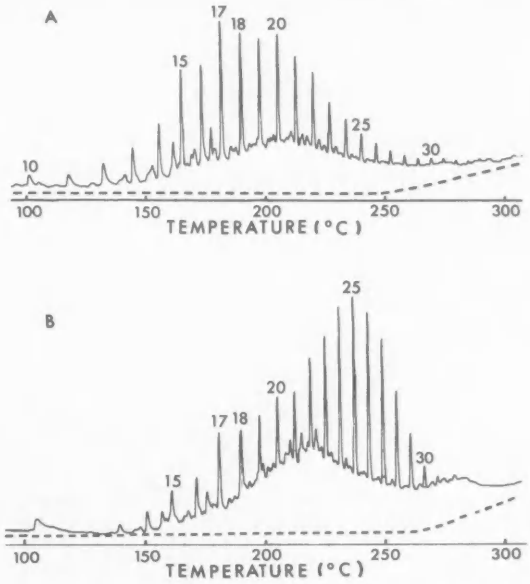


FIG. 1. Gas chromatograms of oil samples. A, Original *Irish Stardust* fuel oil; B, oil from sand obtained 4½ mo after the spill.

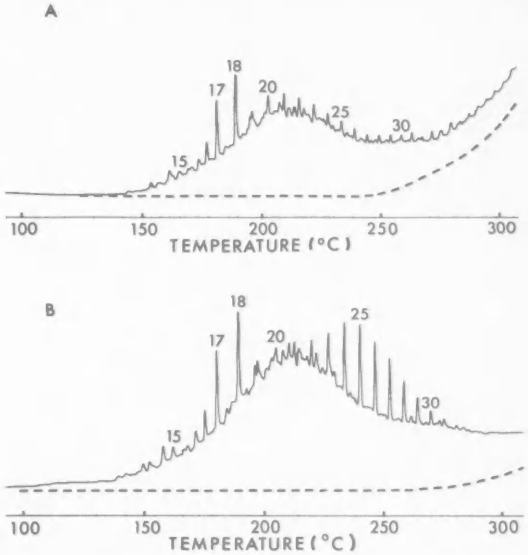


FIG. 2. Gas chromatograms of oil samples. A, Oil from surface gravel; B, oil from surface gravel 2 m from A. Fifth visit, January 26, 1974.

areas. Periwinkles appeared to be moribund or dead, as before, on or near oiled rocks.

The oil had a dull, black, asphalt-like appearance. It still stuck to footwear, however, and had a fresh appearance in crannies and under rocks. It imparted a sheen to water. The gas chromatograms revealed profound changes. Components with retention times less than *n*-eicosane were noticeably truncated, particularly the *n*-alkanes. One chromatogram (Fig. 1B) showed a peculiar anomaly. The *n*-alkanes in the range *n*-docosane to *n*-nonacosane were greatly enhanced, compared with the original oil, over other components in the same retention range.

On August 28, 1973 the oil was still very evident on rock and gravel portions of the beach. It was no longer noticeable in the sand. The amount of oil covering marsh grass was diminished and plants no longer stuck together in clumps. Amphipods, apparently healthy, were found in only slightly increased numbers over the previous visit. No recolonization of the oiled northern rock faces by limpets or littorinids was evident. In contrast, the relatively unoiled southern rock faces still had approximately the same species composition and abundance as was found on the previous two visits.

Exposed oil had a distinct asphalt-like appearance. The advancing tide leached a slick of oil wherever the beach sand was disturbed. Gas chromatograms of oil samples showed an almost complete absence of the *n*-alkanes. The most prominent peaks corresponded to the isoprenoidal alkanes, pristane and phytane.

On January 26, 1974 the signs of oil pollution were no longer obvious in Reserved Bay. At a glance the bay seemed clean and unpolluted. The small stones on the beach and the sand had a normal appearance. The oiled rockweed at the top of the beach had disappeared. Closer inspection revealed some remaining dark oil stains on the rock walls. A thin, unobtrusive, black coating of oil was discernible on some samples of rock and gravel. The oiled marsh grass was reduced in height relative to unaffected stands, but was still alive. Limpets were beginning to recolonize portions of the northern rock face. Periwinkles were found in areas previously devoid of them. Large and small amphipods, all apparently healthy, were found in greatly increased numbers, particularly in the higher tidal levels.

Oil found on the surface of rocks, rock faces, gravel, and grasses had lost the stickiness of fresh oil and oiled objects could be handled without imparting a stain to skin. On the most heavily affected portions of the beach the oil had coagulated to become an immobilizing asphalt-like mortar in the gravel. Oil still leached onto water when the beach sand was disturbed. The chromatograms of samples were essentially unchanged from those of the previous visit. The chromatogram shown in Fig. 2B is exceptional, however, in showing the presence of a much greater amount of *n*-alkanes in the higher molecular weight range than the more typical chromatogram in Fig. 2A. The samples, from which these two chromatograms were obtained, were collected

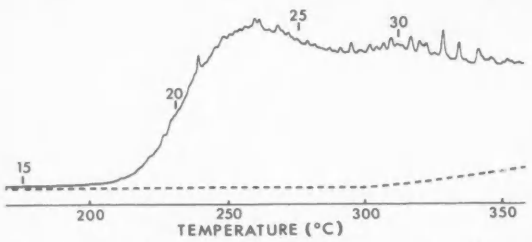


FIG. 3. Gas chromatogram from oil sample found on a rock face at the high tidemark over 4 yr after the spill.

from surface gravel lying only about 2 m apart.

On May 17, 1977 a sixth visit was made to Reserved Bay. A thorough check of the beach was made in search of oil residues. No oil slick was seen to form on the water when the beach was disturbed at the tide's edge. Although no oil residue on samples of stone, gravel, and sand were distinguishable by eye from natural algal cover and mineral inclusions, four of eight samples extracted with carbon disulfide gave a black solution characteristic of residual oil solutions. The gas chromatograms (exemplified in Fig. 3) of these black solutions showed a virtually featureless broad envelope of an unresolved complex mixture of components. Peaks corresponding to *n*-alkanes were absent and those corresponding to pristane and phytane virtually absent. Some noteworthy, albeit small, peaks were discernible, having retention times between those of *n*-octacosane ($nC_{28}$) and *n*-hexatriacontane ($nC_{36}$). This group of peaks is shown enlarged in Fig. 4 together with an enlargement of the corresponding segment of a chromatogram of the fuel from the *Irish Stardust*. Except for the peaks corresponding to *n*-alkanes in the *Irish Stardust* fuel oil, the peaks in the two segments are remarkably similar in shape, relative size, and position. The heights of the non-*n*-alkane peaks with respect to the envelope in the *Irish Stardust* fuel oil are about double the heights of the corresponding peaks of the oil-residue sample.

A number of parameters that reflect weathering effects can be derived (Zafiriou et al. 1972; Blumer et al. 1973) from a series of chromatograms of weathered-oil samples. Some of these parameters relevant to the present study are given in Table 2.

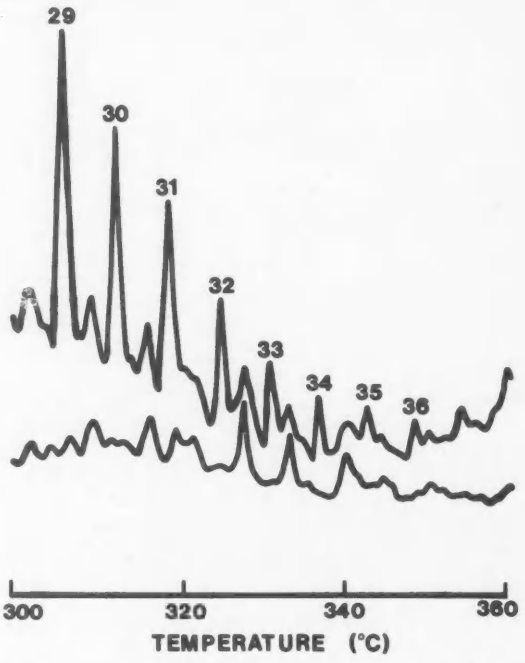


FIG. 4. Comparison of the range (lower trace), $nC_{28}$–$C_{36}$ in the gas chromatogram in Fig. 3 with the same range (upper trace) in a chromatogram of *Irish Stardust* fuel oil.

## Discussion

Several physical, chemical, and biological processes act in concert to degrade oil released into the environment. The interplay of these processes is a function of the nature of oil and the ecosystem into which it has intruded. The present study pertains to a particularly unique dynamic ecosystem, the intertidal zone, which cycles constantly between being marine and terrestrial.

When spilled oil is emergent in the intertidal zone during the day, it is subject to radiant energy from the sun, which together with the wind, promotes evaporation and photooxidation. When oil is submerged or washed by rain, dissolution takes place, particularly of the more soluble oxidation products, some of which may act as emulsifiers to accommodate less-soluble components of the oil. The action of the surf abrades the oil. The buoyancy of seawater and the natural surfactants at the sea surface help to carry it away. As the oil is transformed and dispersed by physical and chemical processes, it is under simultaneous attack by biochemical processes which degrade, transform, and emulsify petroleum hydrocarbons.

The *Irish Stardust* fuel oil can be classed as a No. 5 fuel oil based on its specifications. Because most of its component hydrocarbons have more than 15 carbon atoms, evaporation and dissolution would not be expected to cause dramatic changes in the oil's composition. These related processes would be curtailed particularly in winter, when mean land and water surface temperatures are low and the oil is shielded by clouds and sometimes snow from radiant heating by sunlight. Comparison of the chromatograms of the *Irish Stardust* fuel oil and a typical sample of the oil found 6 wk after the spill supports this supposition. A modest reduction in the relative amounts of the lighter *n*-alkanes by evaporation and dissolution is noticeable. The $C_n^{10\%}$ and $C_n^{50\%}$ values, which should increase with loss of the lighter weight fractions of the unresolved complex

TABLE 2. Characteristics of fuel-oil weathering in an intertidal environment.

| Length of exposure | Location, sample no. | $C_n^{10\%}$[a] | $C_n^{50\%}$ | $nC_{17}$/env | $nC_{17}$/Pr | Pr/Ph | Pr/env[g] |
|---|---|---|---|---|---|---|---|
| — | Fuel tank[b] | 11.6 | 16.5 | 3.1 | 1.9 | 1.3 | 1.6 |
| 5 d | Floating | 11.0 | 16.3 | 3.0 | 1.9 | 1.3 | 1.6 |
| 6 wk | Between rocks | 11.6 | 16.3 | 2.6 | 1.7 | 1.0 | 1.5 |
| 4½ mo | Sand[c] | 14.8 | 18.0 | 1.6 | 1.2 | 0.9 | 1.4 |
| 8 mo | Gravel | 14.2 | 17.0 | 0.4 | 0.3 | 0.8 | 1.2 |
| 1 yr | Gravel, 1[d] | 14.3 | 17.2 | 0.4 | 0.3 | 0.8 | 1.3 |
| 1 yr | Gravel, 2[e] | 14.0 | 17.1 | 0.2 | 0.2 | 0.8 | 1.3 |
| 4 yr | Gravel, 1 | 14.6 | 17.9 | <0.1 | <0.1 | 0.6 | 0.3 |
| 4 yr | Rock face, 2[f] | 18.5 | 20.2 | <0.1 | <0.1 | 0.5 | 0.1 |

[a]Refers to the equivalent *n*-alkane carbon number at which the unresolved envelope reaches the indicated percent of its maximum amplitude (Blumer et al. 1973). [b]Chromatogram in Fig. 1A. [c]Chromatogram in Fig. 1B. [d]Chromatogram in Fig. 2A. [e]Chromatogram in Fig. 2B. [f]Chromatogram in Fig. 3 and 4 (*lower*). [g]"Env" refers to the envelope directly under the indicated compounds; all ratios are height ratios.

mixture, indicate no change in the oil, but the pristane/phytane ratio (Table 1) shows a decrease with time, which is consistent with a very modest amount of evaporation and dissolution of the lighter fractions. A comparatively rapid rise in evaporation and dissolution rates would be expected to occur over the spring months because of the rising mean and maximum surface temperatures in the intertidal area. The $C_n^{10\%}$ and the $C_n^{50\%}$ values rose markedly between the visit on March 9, 1973 and June 5, 1973. This time period corresponds to spring in southwestern British Columbia. After the rapid rise, the indices of evaporation and dissolution become stable within a relatively small degree of variability. One sample, which was obtained from gravel 4 yr after the spill, also does not show an appreciable change in these indices. The other sample, which was obtained from a rock face at the high tidemark, gives $C_n^{10\%}$ and $C_n^{50\%}$ values that represent percentage increases from previously obtained values about equal to the spring increases in 1973. These two samples clearly illustrate that location may have a profound effect on fate of an oil sample in the environment. The sample from the rock face was exposed to direct sunlight and wind for 4 yr. During this time the gravel sample may have been protected from both.

The chromatographic properties (Fig. 1B) of the anomalous sample obtained on the third visit leave its source in some doubt. The ratios of the high molecular weight *n*-alkanes to their concomitant envelope compounds range up to 3 times greater than they are in the *Irish Stardust* fuel oil. Pelagic "tar balls," which are found mainly near tanker routes, have a similar composition (Butler et al. 1973; Green 1976; Wong et al. 1976) and are thought to be formed during cleaning operations from the wax sludge that settles from crude oils on long sea voyages (Brunnock et al. 1968; Ramsdale and Wilkinson 1968). Surveys (Green 1976) have shown, however, that the beaches and waters between the British Columbia mainland and Vancouver Island are virtually free of such residues. The *Irish Stardust* is the most plausible source of the anomalous Reserved Bay sample assuming that its fuel tanks contained a wax residue, some of which was lost in the accident. A less-attractive alternative explanation would require wax precipitation and selective removal of the dewaxed component of the oil once it was on the beach. Microbial lengthening of low molecular weight *n*-alkanes could produce an oil residue having a very large concentration of high molecular weight *n*-alkanes. Elongation of straight-chain fatty acids followed by decarboxylation to *n*-alkanes takes place in some plants (Kolattukudy 1976). Conversion of *n*-alkanes in the fuel oil almost exclusively through a pathway involving elongation–decarboxylation would be required, however, to produce the large *n*-alkane/envelope ratios observed. Such an exclusive pathway for microorganisms does not seem likely.

A wax-sludge derivation could also account for the sample whose chromatogram is shown in Fig. 2B. Alternatively, a less rapid microbial degradation of the higher molecular weight *n*-alkanes compared with the lower molecular weight *n*-alkanes (Kator 1973; Mechalas et al. 1973) could account for the sample. The sample whose chromatogram is shown in Fig. 2A was found <2 m away, however, and is less severely biodegraded as suggested by its higher pristane/envelope and *n*-heptadecane/pristane ratios. Assuming a different microbial population in two similar, close-by sites seems less tenable than assuming that the two samples were different in the beginning.

The more rapid loss of *n*-alkanes with respect to the isoprenoids, pristane and phytane, and the envelope compounds is consistent with microbial degradation (Kator 1973; Mechalas et al. 1973). Microbial degradation also appears to be the primary cause of the more rapid loss of pristane and phytane with respect to the envelope compounds. In the first few months of the spill the effects of microbial degradation on pristane and phytane are masked by dissolution and evaporation, as the larger percentage decrease in the pristane/phytane

ratio (Table 2) compared with the percentage decrease in the pristane/envelope ratio suggests (Ehrhardt and Blumer 1972). The subsequent large percentage decrease in the pristane/envelope ratio and the small percentage decrease in the pristane/phytane ratio show that pristane and phytane are removed at a similar rate, which is much greater than the rate of removal of pristane's concomitant envelope compounds. These compositional changes are consistent with microbial degradation, but not with photooxidation, for example, which would be expected to lead to an increase in the pristane/envelope ratio by selective removal of the aromatic compounds in the envelope (Hansen 1975).

Cycloalkanes (alicyclic hydrocarbons), among hydrocarbons, would seem to be the most resistant to the combined actions of biodegradation and chemical degradation. Most cycloalkanes form part of the unresolved complex mixture, which appears as a hump or envelope in the gas chromatograms of oil samples. Notable exceptions are the pentacyclic triterpanes. These triterpanes have been used as "molecular markers" of oil pollution (Dastillung and Albrecht 1976) and to differentiate among crude oils (Pym et al. 1975). The pentacyclic triterpanes appear prominently in chromatograms of oils and sediment samples from which *n*-alkanes have been removed by molecular seive absorption. Dastillung and Albrecht (1976) have illustrated the resistance of the pentacyclic triterpanes to biodegradation in a chromatogram of an extensively biodegraded crude oil from southwestern France. The chromatogram of the branched and cyclic alkane fraction of this oil, which contains only trace amounts of *n*-alkanes, shows as its only prominent feature a group of peaks corresponding to the pentacyclic triterpanes. The chromatogram in Fig. 3 was obtained from an oil residue sample found on a rock face in Reserved Bay more than 4 yr after the *Irish Stardust* ran aground. The prominent feature in this chromatogram is a suite of peaks in the $nC_{28-36}$ range. It is tempting to speculate that these peaks correspond to the pentacyclic triterpanes in the original oil. The non-*n*-alkane portions of the pentacyclic triterpane regions of chromatograms (Fig. 4) of the rock-face residue sample and the *Irish Stardust* fuel oil are similar enough to suggest that the former is derived from the latter. Unfortunately, comparative examinations of the pentacyclic triterpane region in a representative No. 6 fuel oil reveals that it too is similar enough to be a precursor of the rock-face oil-residue sample. Nevertheless this region in two crude oils, Prudhoe Bay and Peace River, is different enough to exclude them as precursors. Although the source of the rock-face residue sample cannot be established by low-resolution chromatography alone, there would seem to be little doubt that all residue samples found in Reserved Bay on the sixth visit have the *Irish Stardust* fuel oil as their source. The bay is remote from human habitation. No oil spills of any significance that might have been washed into the bay have been reported since the *Irish Stardust* accident. As noted before, the waters and beaches between the mainland and Vancouver Island are virtually free of "tar ball" contamination. Finally, the same "pentacyclic triterpane" pattern was found in chromatograms from samples at widely separated locations in Reserved Bay.

In summary, this study has revealed some expected and some unexpected aspects of the fate and effect of oil spilled in an intertidal zone. Initially, as anticipated, the oil spill adversely affected the intertidal biota. As far as could be determined in the modest biological study, the intertidal biota were well on the road to recovery a year after the spill, by which time the ecosystem had cleansed itself of most of the oil. As expected, evaporation and dissolution accounted for the initial rapid changes in the composition of the spilled oil. These processes proceeded much more rapidly with the approach of summer than they did in winter. The extent to which these processes affected an oil sample depended upon its location. Biodegradation proceeded most rapidly with the *n*-alkanes, followed by pristane and phytane, and then the non-*n*-alkane components in the $nC_{28-36}$ range and the unresolved complex mixture. It was not anticipated that oil samples would be found that seem to be derived from a waxy sludge deposited in the *Irish Stardust* fuel tanks. It was somewhat surprising to find 4-yr-old exposed oil samples still showing in gas chromatograms prominent resolved components, tentatively identified as pentacyclic triterpanes. It was more surprising that the distribution pattern of these components remained very similar to that of the original oil. Oil spill source identification parameters based on these components would apparently be relatively invariant over long periods of time. In this regard a quote from one of Max Blumer's papers (Ehrhardt and Blumer 1972) is appropriate: "For the source identification of hydrocarbons from organisms or from oil spills one will use those parameters which are most resistant to the effects of environmental alteration."

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# Residues of Bunker C Oil in Chedabucto Bay, Nova Scotia, 6 Years After the *Arrow* Spill[1,2]

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KEIZER, P. D., T. P. AHERN, J. DALE, AND J. H. VANDERMEULEN. 1978. Residues of Bunker C oil in Chedabucto Bay, Nova Scotia, 6 years after the *Arrow* spill. J. Fish. Res. Board Can. 35: 528–535.

The *Arrow* spill in February 1970 heavily oiled approximately one half of the 600 km shoreline of Chedabucto Bay, Nova Scotia. An extensive field survey and chemical analysis of sediment samples for aliphatic and polycyclic aromatic hydrocarbons identified only a few locations where *Arrow* Bunker C remained in the intertidal and sublittoral sediments. The upper intertidal zones of Rabbit, Crichton, and Durell islands remain covered with an oil and sediment mixture of a "pavement-like" consistency. Several areas showed visual and chemical evidence of recent spills during the survey period. All sublittoral sediment samples contained hydrocarbons of petroleum origin. The distribution of the most highly contaminated sublittoral sediments suggests either reentry of stranded oil into the water column and into the sublittoral sediments or contamination from shipping and fishing vessels. Concentrations in the sublittoral sediments are below those found toxic to benthic organisms. An estimation of the amount of Bunker C remaining in Chedabucto Bay is impossible due to the patchy distribution, contributions of more recent spills, and the absence of adequate control sites.

*Key words:* Bunker C, oil spill, Chedabucto Bay, gas–liquid chromatography, fluorescence spectroscopy, *n*-alkanes, polycyclic aromatic hydrocarbons

KEIZER, P. D., T. P. AHERN, J. DALE, AND J. H. VANDERMEULEN. 1978. Residues of Bunker C oil in Chedabucto Bay, Nova Scotia, 6 years after the *Arrow* spill. J. Fish. Res. Board Can. 35: 528–535.

A la suite du déversement de l'*Arrow* en février 1970, environ la moitié des 600 km de ligne de rivage de la baie Chedabouctou, Nouvelle-Ecosse, fut recouverte d'une épaisse couche de fuel-oil. Un relevé détaillé sur le terrain et l'analyse chimique des sédiments en vue d'y déceler la présence d'hydrocarbures aromatiques aliphatiques et polycycliques ne révélèrent que quelques endroits où il restait encore du fuel de soute C de l'*Arrow* dans les sédiments intertidaux et sublittoraux. Les zones intertidales supérieures des îles Rabbit, Crichton et Durell sont encore recouvertes d'un mélange de fuel-oil et de sédiments à consistence de « pavage ». Lors du relevé, on a pu constater à l'oeil et à l'analyse chimique les preuves de déversements récents. Tous les échantillons de sédiments sublittoraux contiennent des hydrocarbures d'origine pétrolière. La répartition des sédiments sublittoraux les plus fortement contaminés suggère soit 1 réentrée de pétrole échoué dans la colonne d'eau et dans les sédiments sublittoraux, soit la contamination provenant des navires de transport et des bateaux de pêche. Les concentrations trouvées dans les sédiments sublittoraux sont inférieures à celles qui sont toxiques pour les organismes benthiques. Il est impossible d'estimer la quantité de fuel de soute C qui demeure dans la baie Chedabouctou à cause de sa distribution irrégulière, des apports de déversements ultérieurs et de l'absence d'endroits témoins adéquats.

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THE Liberian tanker *Arrow*, carrying a cargo of 108 000 barrels ($1.75 \times 10^6$ L) of Bunker C fuel oil, ran aground on Cerberus Rock in Chedabucto Bay, Nova Scotia, on February 4, 1970. Over the next several days, an estimated two thirds of her cargo was released into the Bay. Oil slicks, driven by wind, currents, and tides, coated more than one half of the 600 km of shoreline (Anon. 1970). Large quantities of oil in the form of surface slicks and oil droplets in the water column were swept from the Bay by tides and currents (Forrester 1971). Concentrations of total oil in the water column were as high as 100 ppb in May 1970 (Levy 1971).

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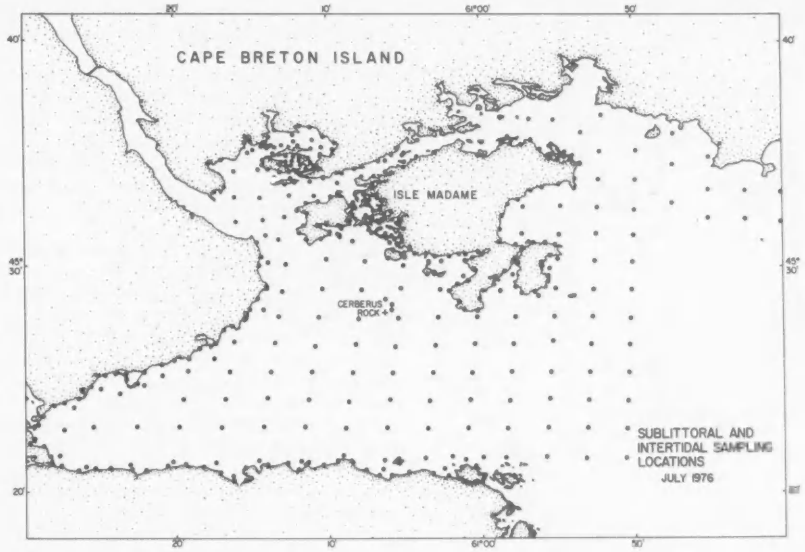


FIG. 1. Location of intertidal and sublittoral sampling stations in Chedabucto Bay, N.S.

By April 1971 concentrations of oil in the water column had dropped to a background level of $<2$ ppb (Gordon and Michalik 1971). By October 1973 much of the oiled shoreline had been cleaned by natural forces (Owens and Rashid 1976). Physical and chemical analyses of stranded oil suggested that the remaining oil would persist for many years (Rashid 1974; Vandermeulen and Gordon 1976). In 1975 Vandermeulen and Gordon (1976) studied the dynamics of oil movement in a lagoon contaminated with *Arrow* Bunker C and concluded that the areas of prime interest were the sediments and interstitial waters.

As part of a comprehensive study designed to evaluate the long-term effects of the *Arrow* spill, we have conducted an extensive survey of surficial sediments, intertidal and sublittoral, of Chedabucto Bay in July 1976. Using a variety of methods, we analyzed sediments for petroleum hydrocarbons in order to determine the distribution and chemical fate of the remaining spilled oil.

## Materials and Methods

### SAMPLING

*Sublittoral* — A van Veen grab was used to obtain samples of surficial sediment from 137 of the 165 locations shown in Fig. 1. The Strait of Canso area was not sampled due to the high probability of petroleum pollution from ship traffic and industry in this area. It was not possible to obtain samples at some locations due to the rocky nature of the substrate. The grab was thoroughly cleaned to remove grease and oil deposits before use and samples were removed from the middle of the grab to minimize the probability of contamination. Subsamples were placed in glass jars and sealed with foil-lined caps.

*Intertidal* — Sampling points were located approximately every 1.5 km along the shore (Fig. 1), and more frequently in areas where oil was still visible. Each of the 266 locations was surveyed for about 100 m on either side of the sampling point and the occurrence of any visible oil residues in the intertidal zone was noted. A mixed-sediment sample from the top 10 cm was obtained by removing a shovel of sediment and then sampling from the exposed surface opposite the shovel. Samples, collected from low, mid-, and high tide at each location, were placed in glass jars with foil-lined caps.

All samples, sublittoral and intertidal, were collected between July 5 and July 21, 1976 and frozen at $-20°C$, within 6 h of collection. Sample containers and foil liners had been prerinsed with redistilled methylene chloride.

One intertidal site was sampled more intensively to determine the composition and distribution of hydrocarbons in the intertidal zone. The study site, an oiled beach in Moussiliers Passage, has been used in previous studies (for full description see Vandermeulen and Gordon 1976). It is a gently sloping beach, with sand to gravel at the high tide line changing to fine sand and silt along the low tide line, and forms part of Haddock Harbour, a large low-energy lagoon. A 2–3-cm thick tar layer lies along the upper high tide line.

Sediment samples were collected in duplicate from high, mid-, and low tide lines, in April 1976 and June 1977. On the first date, samples were collected in glass jars; on the second, cores were taken and frozen within 3 h.

### ANALYTICAL PROCEDURES

Samples were partially thawed and subsamples were placed in preextracted glass Soxhlet thimbles and extracted for 18 h with 250 mL of methanol–benzene (2:3 vol/vol) (Fig. 2). After cooling, the extract was washed with 1N HCl saturated with NaCl and the benzene layer was separated. The aqueous layer was extracted twice with 75

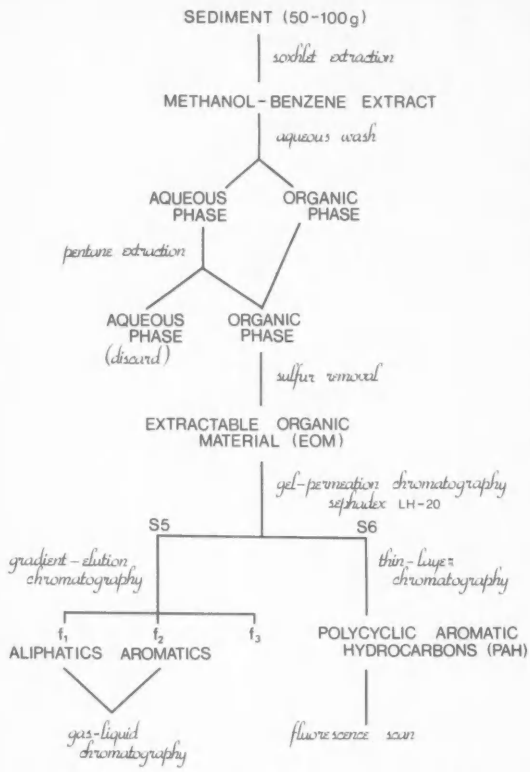


FIG. 2. Flow chart of analytical procedure. Details are given in the text.

mL of pentane and the combined benzene and pentane extracts were washed again with the acidic saturated salt solution. The organic layer was separated and dried over anhydrous $Na_2SO_4$ overnight. The extract was decanted into a Kuderna-Danish concentrator and reduced to 10 mL in volume. After sulfur removal (Blumer 1957) an aliquot was evaporated to dryness and weighed on an electrobalance to determine the concentration of extractable organic material (EOM).

The sample, or an aliquot (maximum of 20 mg EOM), was evaporated almost to dryness under a stream of nitrogen and the benzene was replaced with isooctane. Tetrahydrofuran (THF) was used to elute the sample from a column of 5 g of Sephadex LH-20 (12 × 170 mm). A fraction (S5) containing the *n*-alkanes and another fraction (S6) containing a group of polycyclic aromatic hydrocarbons (PAH) including benzo[*a*]pyrene (Hsieh et al. 1969) were isolated.

Separation of the PAH's from S6 was carried out on a cellulose plate dipped in 20% dimethylformide (DMF) in diethyl ether and developed with isooctane (White and Howard 1967). Aliquots of the PAH fraction from S6 of *Arrow* Bunker C samples and benzo[*a*]pyrene were spotted with the sediment extracts. The developed plate was observed under long wavelength ultraviolet light and then scanned at excitation/emission wavelengths of 271/362 and 365/445 nm on a Perkin-Elmer MPF-2A fluorescence spectrophotometer equipped with the thin-layer scanning accessory. Care was taken at all steps to minimize exposure of the plate to light.

Fractions S5 and S6 from the gel-permeation chromatography were concentrated under a stream of nitrogen and the THF was replaced with isooctane. Samples were then chromatographed on a column of alumina on silica gel (1:1 wt/wt, 5 × 70 mm) eluting with pentane ("aliphatics"), 10 and 20% benzene in pentane ("aromatics"), and benzene. Aliquots of each fraction were evaporated to dryness and weighed.

Aliquots of aliphatic fractions were injected onto a 15 m × 0.5 mm ID stainless steel SCOT column which was held at 100°C for 1 min, then programmed at 8°C/min to 280°C and held. Carrier-gas flow was at a constant pressure of 0.2 kg/cm² (~4 mL He/min at 100°C). Core samples were analyzed on a 25-m OV-101 glass capillary column. The injector, an MS-41 sampling accessory, was at 250°C; the flame ionization detector was at 350°C. Chromatograms were recorded and peak areas determined with a Hewlett-Packard 3380A Integrator. Areas for the unresolved complex mixture (UCM) were determined with a planimeter and concentrations determined by calibration with the UCM for a sample of *Arrow* Bunker C.

All solvents were reagent grade, redistilled in glass, except pentane which was practical grade, redistilled twice in glass. Thin-layer plates were 250 μm cellulose MN300 obtained from various suppliers. Silica gel was Hi-Flosil, 60/200 mesh, activated at 250°C for 18 h; alumina was Alcoa alumina F-20, 80/200 mesh, activated at 400°C for 18 h. Both supports were deactivated with 5% water (wt/wt). Activated copper, $Na_2SO_4$, silica gel, and alumina were all preextracted with solvent before use.

## Results and Discussion

### Intertidal Sediment Samples — Visual Observations

Oil, presumably from the *Arrow*, still remains in the intertidal sediments in many areas of Chedabucto Bay. On the southern and western shores of the Bay, only the area around Durell Island remained heavily oiled (Fig. 3). Oil mixed with sand, gravel, and rocks in a "pavement-like" consistency covers the upper half of the intertidal zone in most of the sheltered locations in this area. There was also evidence of recent spills of light fuel oils. There is considerable ship movement in the area by inshore and offshore fishing vessels to and from the local fish plant at Canso which may be responsible for these spills. Owens and Rashid (1976), based on aerial observations, reported almost complete removal of oil from these shores by October 1973, the extent of oiling reported for the Durell Island area being somewhat less than noted in July 1976. The method of observation and/or accumulation from more recent spills may account for this difference.

The northern shore was the most heavily oiled area at the time of the *Arrow* spill. In July 1976, only an occasional patch of oil remained on the exposed northern coast with the exception of Crichton Island, the adjacent shoreline of Bonhomme Cove and the In-

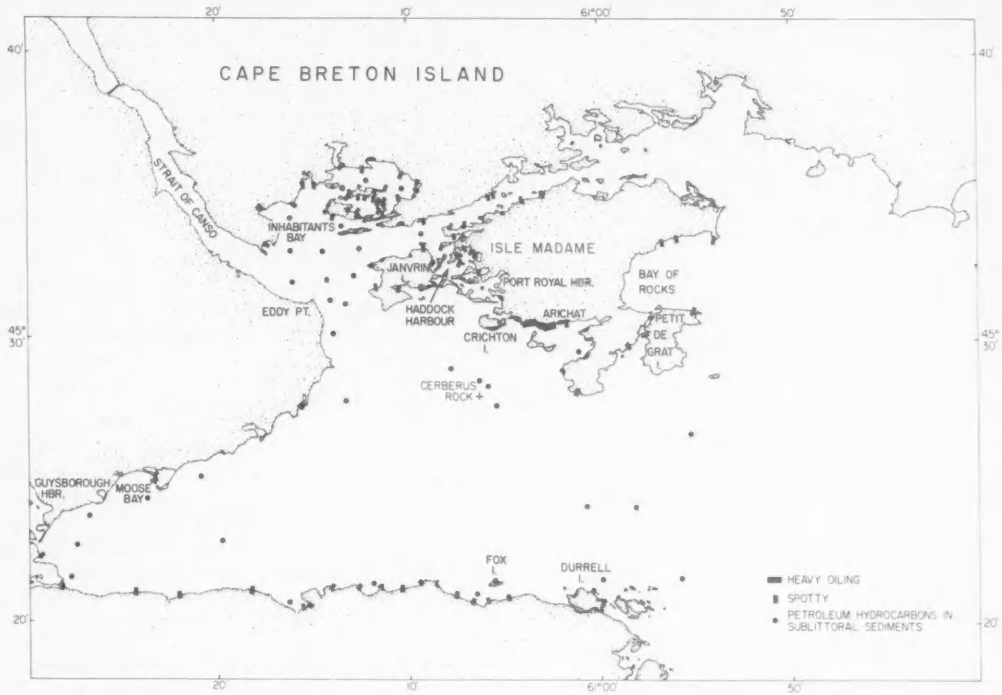


FIG. 3. Extent of oiling in intertidal and sublittoral sediments of Chedabucto Bay, July 1976.

TABLE 1. Concentrations of "oil" in intertidal sediments from Moussiliers Passage based on gas-chromatographic analysis. T1 and T2, transects 1 and 2.

| | | μg Bunker C/g sediment[a] | | |
|---|---|---|---|---|
| | | | June 1977 | |
| Location | Depth (cm) | April 1976 | T1 | T2 |
| High tide | 2–5 | 67 | 110 | 24 |
| | 7–11 | 625 | 148 | 26 |
| | 12–15 | 1200 | 43 | 19 |
| Mid-tide | 2–5 | 106 | 36 | 129 |
| | 7–11 | 1280 | 25 | 145 |
| | 12–15 | 27 | 12 | 41 |
| Low tide | 2–5 | 155 | 442 | 821 |
| | 7–11 | 10 | 15 | 12 |
| | 12–15 | 7 | 41 | 11 |

[a]Based on the area of the UCM using *Arrow* Bunker C fuel oil as a standard.

habitants Bay area (Fig. 3). In the sheltered areas of Haddock and Port Royal harbors, there is little oil evident on the surface of the sediments; however, oil mixed with coarse sand and gravel was found in layers several centimetres thick, 5–10 cm below the surface in several locations. This oiled layer often extended from high to low tide but tended to be very patchy. It is therefore impossible to estimate the amount of oil that may be incorporated into the sediments of the area. Aerial observations in 1973 (Owens and Rashid 1976) did not reveal the presence of any oil in this area, although it had been heavily oiled at the time of the spill. This area is also frequented by small fishing and pleasure boats which may be responsible for some of the oil residues.

The shores of Rabbit Island in Inhabitants Bay and the adjacent point on the mainland are visibly the most heavily oiled areas remaining in Chedabucto Bay. "Pavement," up to 15 cm thick, covers much of the upper half of the intertidal zone and on hot days oily films spread out among the rocks onto the waters of the Bay. The extent of oiling on the remaining shoreline and the other islands of Inhabitants Bay and Inhabitants Harbour has diminished greatly since October 1973. Only the eastern and western ends of Evans Island remain heavily oiled, while only scattered patches of "pavement" remain on the rest of the shoreline. Occasionally, deposits of a subsurface oil and sand mixture were uncovered in this area, but as in the Haddock and Port Royal Harbour area the distribution was patchy.

Concentrations of Bunker C fuel oil, based on

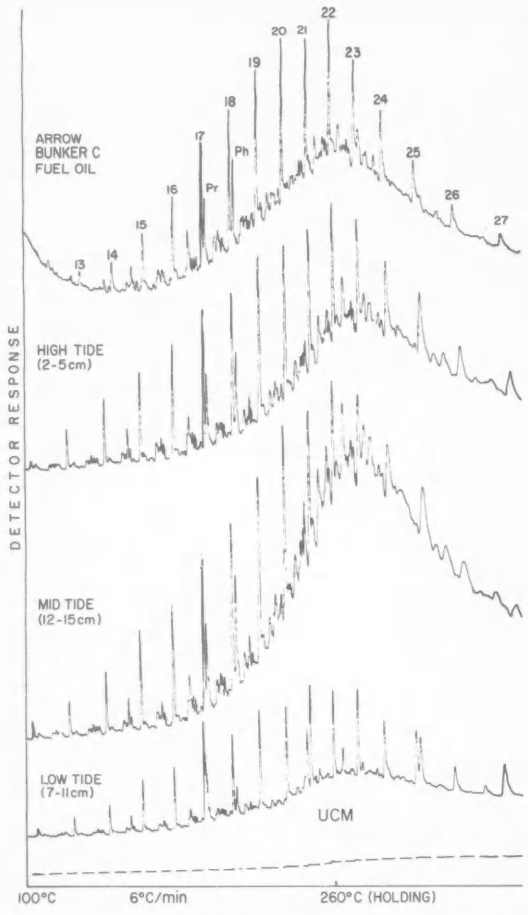


FIG. 4. Gas-liquid chromatograms of the aliphatic fraction of *Arrow* Bunker C fuel oil and sediment extracts from Moussiliers Passage. The broken line is a procedural blank; numbers above peaks indicate the number of carbons in the corresponding *n*-alkanes; Pr is pristane and Ph is phytane.

measurement of the UCM, varied by 2 orders of magnitude at the Moussiliers Passage study site (Table 1). The data suggest removal of the "oil" from the high tide zone with accumulation in the low tide sediments; however, the composition of the saturate fraction suggests another explanation for this apparent change. In April 1976 chromatograms of the saturate fraction revealed only a few minor resolved peaks on a large unresolved envelope, typical of a weathered residual fuel oil. Little difference was observed among the samples. However, samples collected in June 1977 were quite different. Many of the chromatograms (Fig. 4) exhibited a series of *n*-alkanes in the $nC_{14}$–$nC_{22}$ range superimposed on the unresolved envelope, a pattern similar to that of a fresh No. 2 fuel oil. This is a definite indication that a light fuel oil had been spilled in the study area sometime near the sampling date. Therefore more than one spill of fuel oil has probably contaminated this area since the grounding of the *Arrow*. Once a crude or fuel oil has weathered, the unresolved envelope that remains is no more characteristic of *Arrow* Bunker C than any other oil. Because the source of petroleum-derived hydrocarbons in the sediments is not only *Arrow* Bunker C, it is impossible to evaluate the extent of weathering and the mobility of the stranded oil on the beach. This observation must also serve as a caution in interpreting data obtained from other sampling locations, intertidal and sublittoral.

## SUBLITTORAL SEDIMENT SAMPLES

*Gravimetric data* — Concentrations of extractable organic material (EOM) in sublittoral sediments ranged from 5 to 2092 μg/g of dry sediment. The lower values are associated with coarse-grain sediments, the higher values with fine-grain sediments and sediments collected near more heavily populated areas such as Arichat Harbour, Durell Island, and Petit de Grat Inlet. These concentrations of EOM are generally higher than in offshore sediments in this area (Keizer et al. 1978) but of the same order of magnitude as samples from other inshore areas (e.g. Farrington and Tripp 1978). There is no evidence of a massive oiling of the sediment where 25–50% by weight may be extractable organic material.

*Polycyclic aromatic hydrocarbons (PAH)* — The composition of the PAH fraction of petroleum is substantially different from that of biogenic hydrocarbons. The PAH fraction in unpolluted marine sediments is dominated by the unsubstituted hydrocarbons or derivatives with low alkyl content, while in crude and Bunker C fuel oils, hydrocarbons with a greater alkyl substitution dominate (Youngblood and Blumer 1975). No attempt was made to isolate and determine concentrations of individual PAH's. The general composition of the PAH fraction should be an indicator of the presence of petroleum hydrocarbon residues.

Thin-layer chromatography of the PAH fraction of *Arrow* Bunker C fuel oil did not reveal the presence of any single (or series of) dominating hydrocarbon(s) (Fig. 5). A blue streak extending from the origin to the solvent front was apparent under long wavelength UV illumination. Chromatography of the PAH fraction from the sublittoral sediment samples generally revealed a continuous series of multicolored fluorescing bands under UV illumination and a series of peaks in the fluorescence scan (Fig. 5). The band that had an $R_f$ value identical to benzo[*a*]pyrene had a fluorescence emission spectrum identical to the standard. Although differences in the relative intensities of peaks were observed, the same pattern was present in all samples.

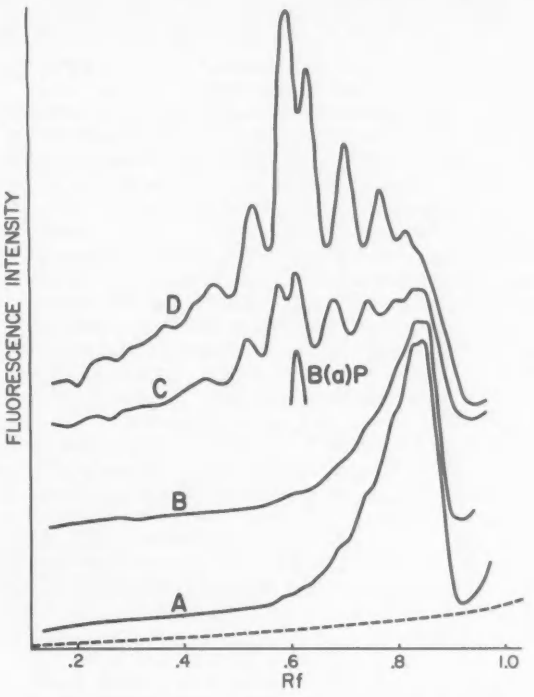


FIG. 5. Fluorescence emission scans of TLC plates of PAH fractions (reverse phase cellulose — 20% DMF in ether developed in isooctane). Excitation wavelength, 365 nm; emission wavelength, 445 nm. A *Arrow* Bunker C fuel oil; B weathered *Arrow* Bunker C fuel oil; C extract from sublittoral sediment containing petroleum residues; D extract from unpolluted sublittoral sediments.

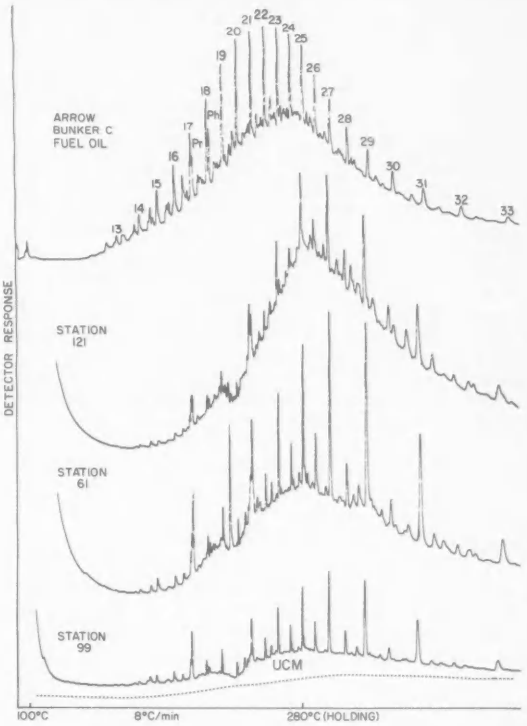


FIG. 6. Gas–liquid chromatograms of aliphatic fractions of *Arrow* Bunker C fuel oil and sediment extracts from Stations 121 (north of Janvrin Island), 61 (north of Durell Island), and 99 (45°25′N, 60°50′W) exhibiting, in order, decreasing degrees of petroleum hydrocarbon contamination. The broken line is the background signal; numbers above peaks indicate the number of carbons in the corresponding *n*-alkanes; Pr is pristane and Ph is phytane. Chromatograms represent approximately the same weight of sediment.

This widespread uniformity in the composition of the PAH fraction of sediment extracts has been observed before (Youngblood and Blumer 1975) and suggests a common source, possibly the anthropogenic combustion of fossil fuels (Hites et al. 1977).

The presence of an underlying blue streak and/or a strong blue band near the solvent front is indicator of the presence of petroleum-derived hydrocarbons in samples. This visible feature was always correlated with small peaks superimposed on a high background in the fluorescence scans. The samples taken at the mouth of the Strait of Canso, near Eddy Point, and just north of Durell Island, had TLC scans identical to Bunker C fuel oil. Both these locations are probably the recipients of chronic pollution from ship traffic.

*Gas–liquid chromatography* — A chromatogram of the aliphatic fraction of a sample of *Arrow* Bunker C fuel oil is shown in Fig. 6. It is characterized by a series of *n*-alkanes and isoprenoids on an envelope due to a UCM of principally naphthenic hydrocarbons (Blumer et al. 1970). Numerous studies (e.g. Atlas and Bartha 1973) have documented the rapid bacterial degradation of the *n*-alkanes, the UCM being much more resistant to degradation.

The aliphatic fraction from all of the sublittoral samples contained a series of *n*-alkanes that had a strong odd-carbon preference (Fig. 6) indicative of a biogenic terrestrial source for these hydrocarbons (Farrington and Meyers 1975). The sample from near the mouth of the Strait of Canso was an exception. It had a carbon preference index (Bray and Evans 1961) of 1.03 for $nC_{16}$–$nC_{30}$ with a maximum concentration at $nC_{25}$, a strong signal for the UCM peaking at $nC_{22}$, and an $nC_{18}$/phytane ratio of 1.35. These are all strong indicators of recent petroleum hydrocarbon input (Farrington and Meyers 1975).

Chromatograms of the aliphatic fraction of all samples had a measurable unresolved envelope. The concentration of this UCM ranged from barely detectable,

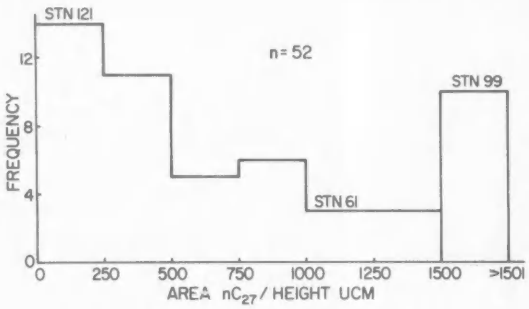


FIG. 7. Frequency distribution of the ratio of the $nC_{27}$ peak area to the height of the UCM at $nC_{27}$. The class to which the chromatograms from Fig. 6 belong is indicated. The probability of oil residues in the samples increases from right to left.

<0.05, to 117 μg/g dry sediment. The shape of the UCM was generally different than that for *Arrow* Bunker C and indicated the loss of some of the lower boiling components (Fig. 6). Most crude and residual fuel oils contain a UCM and the presence of a UCM in marine organism extracts is generally taken as an indicator of the presence of petroleum hydrocarbons (Farrington and Meyers 1975). Farrington and Tripp (1978) have reviewed the possible sources of the UCM in marine sediments and have concluded that the presence of the UCM is a strong indicator of fossil hydrocarbon contamination.

Extracts from these sediments had UCM's that varied considerably in shape and intensity (Fig. 6). A measure of the relative intensity of the UCM was made by comparing the area of the $nC_{27}$ peak relative to the height of the UCM at this point. The frequency distribution of this data is plotted in Fig. 7. Samples that have ratios in the far left of the histogram have a larger UCM relative to the concentration of *n*-alkanes and therefore a higher probability of containing petroleum-derived hydrocarbons than those on the far right. The class to which the chromatograms from Fig. 6 belong is indicated.

Based on the $nC_{27}$ to UCM ratio and the fluorescence TLC scans of the PAH fraction, probabilities were assigned for the presence of petroleum-derived hydrocarbons in a sample. On this basis petroleum-hydrocarbon residues in sublittoral sediment extracts are found principally in heavily traveled areas (Fig. 3) where the source of the residues is complicated by inputs from many sources over long periods of time. On the northern shore, stations with sediment extracts containing petroleum residues are concentrated near the entrance to the Strait of Canso and Cerberus Rock. This may be the result of either a large proportion of the fuel oil spilled from the *Arrow* being swept there by natural forces and/or chronic spills by ships using the Strait of Canso. It is impossible to say whether or not the petroleum residues in the sediment came from the *Arrow*; however, the residues appear to be extensively weathered. The *n*-alkanes have disappeared and the composition of the PAH fraction is significantly different from that of a petroleum product.

Oil may reach the sublittoral sediments by a number of pathways. For a residual fuel oil such as Bunker C, the loss of volatile and soluble components and the attachment of mineral particles can result in an oil residue with a density greater than that of seawater (McAuliffe 1977). In the case of the *Arrow* spill, the density of the surface waters was only 5% greater than that of the spilled oil (Conover 1971). Conover (1971) concluded that zooplankton were responsible for the removal of oil droplets from the water column by ingestion and subsequent sedimentation as oil incorporated with other fecal material. In addition to these methods of sedimentation, stranded oil that has been mixed with intertidal sediments is swept into the water column by wave and tidal action and finds its way to the sublittoral sediments. The distribution of oil-containing sediments and the absence of a petroleum-derived *n*-alkane series favors the last pathway as the major source of petroleum-derived hydrocarbons in the surficial sublittoral sediments of Chedabucto Bay. If it were assumed that all of the extractable organic material from these sediments were *Arrow* Bunker C, the concentrations would still be far below the level at which toxicity to benthic organisms is observed (e.g. Gordon et al. 1978).

## Conclusions

Stranded oil from the tanker *Arrow* has disappeared from most of the shoreline of Chedabucto Bay, 6 yr after the spill. Visual observations and chemical analysis of samples from 600 km of shoreline (including 266 stations) revealed the presence of petroleum hydrocarbons at most locations. However, due to weathering and contamination from recent spills, identification of the residues as *Arrow* Bunker C is impossible.

Only a few isolated spots remain that are visibly contaminated with large quantities of oil identifiable as *Arrow* Bunker C. These are Rabbit, Crichton, and Durell islands, where the upper high tide zones remain covered with a mixture of oil and sediment in a "pavement-like" consistency.

Estimation of the amount of oil buried within the intertidal sediments is hampered by its patchy distribution and re-oiling and contamination from other sources. Chemical analysis of the aliphatic and polycyclic aromatic hydrocarbon fractions of sediment extracts from one location studied in detail (Moussiliers Passage) indicated recent contamination with a light fuel oil. In several other areas there was visual evidence of light-fuel-oil spills during the survey period.

Chemical analysis of the aliphatic and polycyclic aromatic hydrocarbon fractions of 72 of the 137 sublittoral samples collected indicated a variable degree of contribution of petroleum hydrocarbons to the extractable organic material. The samples that contained the

highest concentrations were generally located along the northern shore and in the Strait of Canso area. It is not possible to determine whether *Arrow* Bunker C is the source of these hydrocarbons because of extensive weathering and probable input from recent spills. However, the distribution suggests that the source may be the reentry of stranded oil into the water column and into the sublittoral sediments near the shore.

The main difficulty in a study of this type is our inability to find a control site that has not been contaminated by petroleum hydrocarbons and does not have measurable quantities of petroleum-derived hydrocarbons in its intertidal and sublittoral sediments. Without an appreciation for what "background" levels might be, it is impossible to assess the magnitude of petroleum-hydrocarbon input from a spill.

## Acknowledgments

We thank Mr W. R. Hardstaff, Ms L. Rutley, and the captain and crew of the *Whip-the-Wind* for their assistance in conducting the survey and Dr D. C. Gordon, Jr. for his many helpful suggestions. The Canadian Coast Guard provided helicopter time for preliminary aerial reconnaissance of the study area.

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# Contribution of Chronic Petroleum Inputs to Narragansett Bay and Rhode Island Sound Sediments[1]

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VAN VLEET, E. S., AND J. G. QUINN. 1978. Contribution of chronic petroleum inputs to Narragansett Bay and Rhode Island Sound sediments. J. Fish. Res. Board Can. 35: 536–543.

Sediment cores from Narragansett Bay and Rhode Island Sound have been analyzed for petroleum hydrocarbons and compared with a relatively unpolluted sediment core from the Gulf of Maine. The sediments were analyzed for unbound hydrocarbons, hydrocarbons bound or closely associated with humic substances, and residual hydrocarbons bound or closely associated with the clay mineral or kerogen matrix. Results indicated that in general 90–100% of the hydrocarbons were in the unbound form and could be easily extracted with organic solvents. The petroleum hydrocarbons decreased with depth at all stations. Biogenic hydrocarbons ($n$C$_{25}$, $n$C$_{27}$, $n$C$_{29}$, and $n$C$_{31}$) made up an increasingly greater percentage of the total with increasing depth. The hydrocarbons in the Narragansett Bay sediments and near surface Rhode Island Sound sediments strongly resembled the hydrocarbons previously reported for the Providence River and upper Narragansett Bay. These petroleum-like hydrocarbons were shown to be largely introduced to the river and bay through chronic inputs from a municipal wastewater treatment plant. These hydrocarbons then undergo sedimentation throughout the entire bay and into Rhode Island Sound. Preliminary calculations indicate that over 0.2 million t (tonne) of petroleum hydrocarbons may be transported to the marine environment annually from municipal treatment plants. Most of these hydrocarbons appear to accumulate in estuarine and coastal sediments.

*Key words:* hydrocarbons, petroleum, chronic pollution, Narragansett Bay, sediments

VAN VLEET, E. S., AND J. G. QUINN. 1978. Contribution of chronic petroleum inputs to Narragansett Bay and Rhode Island Sound sediments. J. Fish. Res. Board Can. 35: 536–543.

Nous avons mesuré les teneurs en hydrocarbures de pétrole dans des carottes de sédiment de la baie Narragansett et du détroit de Long Island et les avons comparées avec celle d'une carotte de sédiment relativement non pollué provenant du golfe du Maine. Nous avons déterminé dans les sédiments les hydrocarbures fixés ou étroitement associés aux substances humiques et les hydrocarbures résiduaires fixés ou étroitement associés au minérai argileux ou à la matrice de kérogène. D'après les résultats, de 90 à 100% en général des hydrocarbures sont sous la forme non fixée et peuvent être facilement extraits avec des solvants organiques. Les hydrocarbures de pétrole diminuent en fonction de la profondeur à toutes les stations. Les hydrocarbures biogéniques ($n$C$_{25}$, $n$C$_{27}$, $n$C$_{29}$ et $n$C$_{31}$) constituent un pourcentage croissant du total à mesure qu'augmente la profondeur. Les hydrocarbures dans les sediments de la baie Narragansett et près de la surface des sédiments du détroit de Rhode Island ressemblent beaucoup aux hydrocarbures déjà signalés dans la rivière Providence et le haut de la baie Narragansett. On a démontré que ces hydrocarbures ressemblant au pétrole avaient été en grande partie introduits dans la rivière et dans la baie par l'effluent d'une usine de traitement des eaux usées municipales. Ces hydrocarbures subissent par la suite une sédimentation dans toute la baie et dans le détroit de Rhode Island. Des calculs préliminaires indiquent que plus de 0.2 million t (tonnes métriques) d'hydrocarbures de pétrole peuvent être transportés dans l'environnement marin annuellement en provenance d'usines de traitement d'égouts municipaux. La plupart de ces hydrocarbures semblent s'accumuler dans les sédiments estuariens et côtiers.

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TABLE 1. Summary of estimated petroleum inputs to the marine environment (million tonnes per year).

| SOURCE | SCEP 1970 | Jeffrey 1971 | Porricelli et al 1971 | USCG 1973 | Brummage 1973 | Charter et al 1973 | Duce and Quinn 1973 | Feuerstein 1973 | Storrs 1973 | Wilson 1973a,b | NAS 1975 | Grossling 1976 | Koons and Monaghan 1976 | Smith 1976 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Marine Operation Losses | | | | | | | | | | | | | | |
| LOT tankers | 0.03 | 0.10 | - | | 0.25 | 0.26 | - | - | - | - | 0.31 | | - | |
| Non-LOT tankers | 0.50 | 0.60 | - | | 0.75 | 0.46 | - | - | - | - | 0.77 | 0.41 | - | 1.00 |
| Bilges, Bunkering, and other normal ship operations (all ships) | 0.50 | 0.05 | - | 1.72 | 0.50 | 0.61[a] | - | - | - | - | 0.50 | | - | 0.30 |
| Offshore accidental discharges | | | | | | | | | | | | | | |
| Tanker accidents | 0.10 | | - | | 0.12 | 0.21 | - | - | - | - | 0.20 | 0.22 | - | |
| Accidents, other ships | | 0.20 | - | | 0.02 | 0.14 | - | - | - | - | 0.10 | - | - | 0.35 |
| Pipeline accidents | - | | - | - | - | - | - | - | - | - | - | <0.01 | - | |
| Offshore oil production | 0.10 | 0.15 | - | 0.12 | 0.10 | 0.12 | - | - | - | 0.08 | 0.08 | <0.38 | - | 0.15 |
| Natural marine oil seeps | - | | - | - | - | - | - | - | - | 0.2-6.0 | 0.60 | 0.24-7.00 | 0.60 | 0.60 |
| Atmospheric deposition | (9.0)[b] | - | - | - | - | - | 1.6 | 0.4-0.8 | - | - | 0.60 | - | - | - |
| Land based discharges | | | | | | | | | | | | | | |
| Refineries | 0.30 | 0.30 | - | - | 0.30 | 0.20 | - | - | - | - | 0.20 | <0.62[c] | - | |
| Terminal transfer operations | 0.10[d] | - | - | [e] | 0.01 | 0.03 | - | - | - | - | 0.25[f] | | - | |
| Pipeline accidents | - | - | - | - | | 0.03 | - | - | - | - | - | <0.01 | - | |
| Runoff (urban and river) | | - | | - | | - | - | - | - | - | 1.90 | - | - | |
| Industrial wastes | 0.45 | | | 1.98 | 0.50 | 0.72 | - | - | 0.08 | - | 0.30 | 1.64 | - | 1.30 |
| Automotive wastes | | 0.50 | 4.4 | - | | 1.03 | - | - | - | - | - | 1.03 | - | |
| Aviation wastes | - | | | - | | - | - | - | - | - | - | 0.05 | - | |
| Municipal wastes | - | | | - | | (11.8)[b,g] | - | - | 0.20 | - | 0.30 | - | - | |
| TOTAL | 2.08 | 1.90 | <9.50 | - | 2.55 | 3.81 | - | 2.4-3.8 | - | - | 6.11 | 3.6-11.4 | - | 3.70 |

[a]Includes product tankers, ore and bulk-oil carriers, disposal prior to dry-docking, and tanker barges and bilges.
[b]Not included in total.
[c]Includes onshore oil production accidents.
[d]Includes all nonship accidental spills.
[e]Included in 1.72 million t above.
[f]Includes dry-docking operational spills.
[g]Based on Philadelphia average of 0.0177 tons/(person·yr) extrapolated to a world population of $4 \times 10^9$ and corrected for 30% of total world oil usage by U.S.

THE trend over the past few years has been to address the problem of oil pollution primarily in terms of the effects of direct spills from a point source occurring over a fairly short time frame. In particular, these acute spills result principally from tanker operations and spills. These spills produce the most immediate and obvious environmental effects on local pelagic and benthic communities and consequently have become the subject of the most immediate concern. Before we can construct a global budget of petroleum inputs to the marine environment, however, it becomes necessary to also address the problem of relatively long-term, continuous inputs. These chronic petroleum inputs have, to a large extent, remained uninvestigated. In this paper, we initially review what we know about chronic inputs to the marine environment; then we discuss our approach to investigating one of these inputs and demonstrate the need for further investigations in this and similar areas.

Table 1 shows a summary of several estimates of worldwide petroleum sources and inputs to the marine environment. It can be seen that the list is incomplete and approximate. The source categories can be reduced into four major input areas: (1) oil production operations (including drilling, normal shipping losses, spills, and refinery operations); (2) land-based discharges other than those directly related to oil production; (3) natural oil seeps; and (4) atmospheric deposition. It is generally accepted that oil production and transport op-

erations result in the largest quantity of oil discharged into the oceans. These inputs are the most closely monitored and as a result, show the least variation. Very little work has been conducted on land-based discharges, natural seeps, or atmospheric deposition into the oceans. As a result, the relative contribution of these sources is still fairly obscure.

We have recently completed a study of the chronic input of petroleum hydrocarbons to Narragansett Bay and Rhode Island Sound from a municipal wastewater treatment plant. Initially, a 1-yr background survey was conducted of the hydrocarbons discharged from the Fields Point Sewage Treatment Plant, Providence, Rhode Island. The hydrocarbons in the receiving water of the Providence River, as well as surface sediments and sediment cores from the river, were then investigated to determine the transport mechanisms and fate of these hydrocarbons in the river (Van Vleet and Quinn 1977).

In this report, we found that wastewater treatment plants may discharge 60% of the total petroleum hydrocarbons entering U.S. coastal estuaries annually by direct spills. This conclusion was reached on the basis of an average annual discharge of 2.80 ± 1.31 mg hydrocarbons/L from the Fields Point plant. Whipple et al. (1976) have shown that the discharge from a Trenton, New Jersey treatment plant averaged 1.49 mg/L, while Tanacredi (1977) showed that the average hydrocarbon discharges from four Jamaica Bay, New York area treatment plants ranged from 6.1 to 21.8 mg/L. These studies show that our initial concern over chronic hydrocarbon inputs is warranted and that these concerns are of importance to many coastal communities. The present study was undertaken to investigate the transport and fate of these hydrocarbons into lower Narragansett Bay and Rhode Island Sound coastal waters. Analysis of a relatively unpolluted station from the Gulf of Maine was undertaken for comparison.

## Materials and Methods

Sediment cores for the present study were taken in mid-Narragansett Bay (41°33.3′N, 71°20.0′W), Rhode Island Sound (41°22.7′N, 71°20.3′W), and the Gulf of Maine (43°00′N, 69°56′W) (Farrington et al. 1977). The earlier Providence River cores were analyzed for total hydrocarbons (Van Vleet and Quinn 1977). In this study the sediments from mid-Narragansett Bay were analyzed for bound versus unbound hydrocarbons, while the sediments from the Rhode Island Sound and the Gulf of Maine were analyzed for unbound hydrocarbons, hydrocarbons bound or associated with humic materials, and residual hydrocarbons bound or associated with the clay mineral matrix, kerogen matrix, or residual plant or animal remains as described below.

Each core was subdivided into 5–10-cm sections, the outer 2–3 mm scraped off to remove any contamination from the core liner, and stored in glass jars at −20°C until analyzed. Surface sediments from each core were wet sieved through a 1-mm mesh screen to remove large benthic organisms and detrital material. Wet sieving does not remove significant quantities of hydrocarbons from the sediments (Van Vleet and Quinn 1977; Boehm and Quinn 1978). A small portion of each sediment was analyzed for moisture content for later normalization of the results to dry weight.

Internal hydrocarbon standard ($nC_{20}$) was added to approximately 50–100 g dry weight (100–200 g wet weight) of each sediment section in a 1-L round-bottom flask. Each sediment sample was then refluxed for 2 h with 500 mL of methanol:toluene (70:30) to extract the unbound lipoidal material. After filtering through a Whatman GF/C glass fiber filter (precombusted for 4 h at 450°C), the filtrate was transferred to a separatory funnel. The phases were separated by adding 350 mL of distilled water, the toluene removed, and the aqueous-methanol phase extracted two additional times with 100-mL portions of petroleum ether. The organic phases were combined and evaporated to near dryness under vacuum at 35°C with a rotary evaporator. The unbound organic material was then saponified for 30 min in a 25-mL centrifuge tube under $N_2$ (in a 100°C water bath) using 7 mL 0.5 N KOH in absolute methanol plus 3 mL toluene plus 1.5 mL distilled $H_2O$. After cooling, the phases were then separated by addition of 5.5 mL distilled water, the toluene removed, and the basic aqueous-methanol phase extracted twice more with 5-mL portions of petroleum ether. The organic phases were then combined and evaporated to dryness.

The Narragansett Bay core sediment (after filtering off the organic solvents as above) was again transferred to a 1-L round-bottom flask. After addition of $nC_{20}$ internal standard, the sediment was saponified under reflux for 2 h with 350 mL of 0.5 N KOH in absolute methanol plus 150 mL toluene plus 50 mL distilled $H_2O$ to remove the bound lipoidal material. (Previous analyses [Van Vleet and Quinn 1977] have shown that virtually no $nC_{20}$ or unbound hydrocarbon material remains after the unbound material is removed as above.) After cooling, the samples were filtered through a precombusted Whatman GF/C glass-fiber filter and the filtrate was transferred to a separatory funnel. The phases were separated by adding 300 mL of distilled $H_2O$, the toluene drawn off, and the basic aqueous-methanol phase extracted twice more with 100-mL portions of petroleum ether. The organic phases were then combined and evaporated to dryness.

The sediments from Rhode Island Sound and the Gulf of Maine (after filtering off the organic solvents containing the unbound lipoidal material) were transferred to a 1-L Teflon bottle. Two hundred millilitres of 0.5 N NaOH were added and the sample was shaken for 2 h at room temperature to extract the humic substances. The sample was then filtered through a precombusted Whatman GF/C glass-fiber filter. The sediment was transferred to a 1-L round-bottom flask and saponified to remove the remaining bound lipoidal material as above. The NaOH solution containing the humic substances was transferred to a 1-L round-bottom flask, internal standard ($nC_{20}$) plus 250 mL methanol was added, and the humic substances were saponified under reflux for 2 h. After cooling, the basic aqueous methanol phase was transferred to a separatory funnel and the nonsaponifiable lipoidal material was extracted 3 times with 100-mL portions of petroleum ether. The petroleum ether phases were combined and evaporated to dryness under vacuum.

All samples were then passed through 10 × 0.55 cm glass columns containing 1 g of 5% deactivated alumina over 0.3 g of activated copper powder. Each sample was

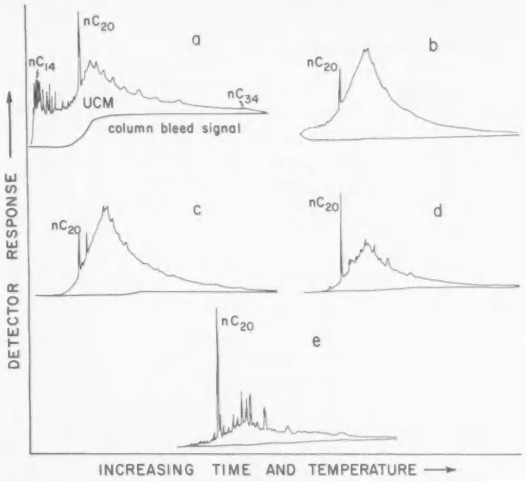


FIG. 1. Unbound hydrocarbon chromatograms from surface sediments of (a) Fields Point (Station 2, Providence River, Van Vleet and Quinn 1977), 0–10 cm (total hydrocarbons), (b) Providence River, Station 3, 0–10 cm, (c) mid-Narragansett Bay, 0–10 cm, (d) Rhode Island Sound, 0–8 cm, and (e) Gulf of Maine, 1–2 cm. Samples analyzed by 8% FFAP on Chromosorb W (HP), 2 m × 2.2 mm ID stainless steel columns. Chromatographic conditions given in text.

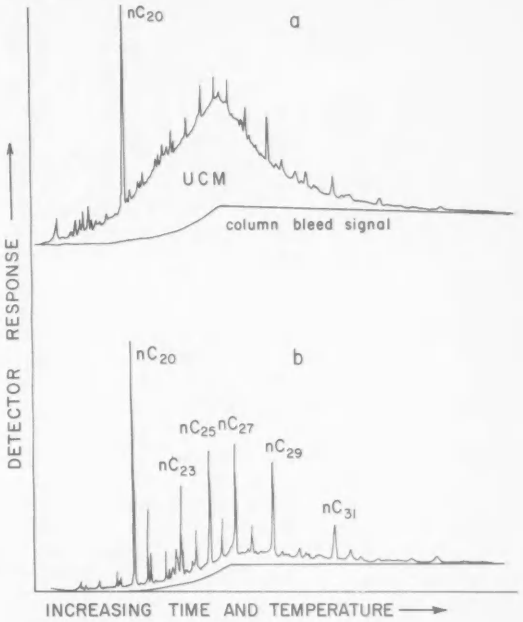


FIG. 2. Unbound hydrocarbon chromatograms from Rhode Island Sound sediment core: (a) 0–8 cm, (b) 28–38 cm. Samples analyzed on 15 m × 0.25 mm ID OV-101 glass capillary column. Chromatographic conditions given in text.

charged to the column in 1 mL of 95:5 petroleum ether:toluene and the hydrocarbons were eluted with an additional 5 mL of 95:5 petroleum ether:toluene. This column procedure removed most of the polar, nonhydrocarbon organic material and elemental sulfur prior to thin-layer chromatography (TLC). The column eluate was evaporated to near dryness and the residue was applied to a (20 × 20 cm) predeveloped 0.37-mm Silica Gel-G TLC plate which was then developed in 97:3:1 petroleum ether:ethyl ether:ammonium hydroxide to isolate the hydrocarbons. The entire plate was visualized by spraying with bromothymol blue indicator and the hydrocarbon band corresponding to separately chromatographed standards of $n$C$_{26}$ and phenanthrene was scraped and extracted from the silica gel with $CH_2Cl_2$. The methylene chloride was then evaporated and the residue redissolved in carbon disulfide. Each hydrocarbon sample was analyzed by gas–liquid chromatography (GLC) with a Hewlett-Packard Model 5711 gas chromatograph equipped with dual-flame ionization detectors and dual 2 m × 2.2 mm ID stainless steel columns containing 8% FFAP on 80/100-mesh Chromosorb W (HP) or a single 15 m × 0.25 mm ID OV-101 glass capillary column (Quadrex Corp.). The columns were temperature programmed from 100 to 240°C at 8 and 4°C/min, respectively. The hydrocarbons measured eluted between $n$C$_{12}$ and $n$C$_{31}$. The chromatograms were quantified by comparing their areas, measured by planimetry, with the areas of the $n$C$_{26}$ internal standard peaks. Corrections were made for any naturally occurring $n$C$_{26}$. Procedural blanks were determined by carrying all solvents and internal standards through the entire analysis in the absence of sediments.

## Results and Discussion

Figure 1 shows the hydrocarbon chromatograms of surface sediments from five areas — (a) directly adjacent to the Fields Point sewage treatment plant outfall; (b) Station 3, lower Providence River (Van Vleet and Quinn 1977). (This sediment comes from a surface grab sample at Station 3 and is not from the sediment core previously reported.); (c) mid-Narragansett Bay; (d) Rhode Island Sound; and (e) Gulf of Maine. The values for total hydrocarbons found at each station are reported in Table 2. In general, the total hydrocarbons decreased with distance away from the Fields Point plant and with depth in each core. The decrease is from 5410 μg/g in surface sediments adjacent to the effluent outfall to a low value of 2–3 μg/g in the bottom sections of the Rhode Island Sound and Gulf of Maine cores. The value of 9.7 μg/g found at 61–65 cm in the Gulf of Maine core is believed to be anomalous due to contamination. The chromatogram of total hydrocarbons for this sample much more closely resembled the blank chromatograms than the sedimentary hydrocarbon chromatograms directly above and below this section. The values we report are in good agreement with the values reported by Boehm and Quinn (1978) for total hydrocarbons in the Rhode Island Sound core (Station 17) and by Farrington et al. (1977) for total hydro-

TABLE 2. Concentrations of total hydrocarbons found in northwest Atlantic coastal sediments.

| Depth (cm) | Providence River[a] Stn. 1 | Stn. 2 | Stn. 3 | Stn. 4 | Narragansett Bay | RI Sound[b] | Gulf of Maine[c] |
|---|---|---|---|---|---|---|---|
| 0–10 | 1410[d] | 5410 | 450 | 570 | 287 | 24.9 | 19.0 |
| 10–20 | 1230 | 4560 | 510 | 470 | 323 | 17.8 | 7.2 |
| 20–30 | 540 | 4460 | 330 | 20 | 172 | 7.7 | 4.2 |
| 30–40 | 520 | 1480 | 20 | 20 | 35 | 1.9 | 2.8 |
| 61–65 | — | — | — | — | — | — | 9.7[e] |
| 85–88 | — | — | — | — | — | — | 2.7 |

[a]Values quoted from Van Vleet and Quinn (1977).
[b]Depths sampled for Rhode Island Sound core are 0–8, 8–18, 18–28, and 28–38 cm.
[c]Depths sampled for Gulf of Maine core are 1–2, 9–11, 21–25, 37–41, 61–65, and 85–88 cm.
[d]All values expressed as micrograms total hydrocarbon per gram dry weight sediment.
[e]Suspected contamination of sample.

TABLE 3. Percentage of total hydrocarbons found in each fraction (followed by percentage of these hydrocarbons present as resolved components).

| | Narragansett Bay | | Rhode Island Sound | | | Gulf of Maine | | |
|---|---|---|---|---|---|---|---|---|
| | Unbound | Bound | Unbound | Humic | Residual | Unbound | Humic | Residual |
| 0–10 | 84 (4) | 16 (4) | 100 (5) | 0 (–) | 0 (–) | 100 (25) | 0 (–) | 0 (–) |
| 10–20 | 96 (4) | 4 (5) | 86 (13) | 6 (38) | 8 (24) | 100 (20) | 0 (–) | 0 (–) |
| 20–30 | 94 (13) | 6 (9) | 100 (27) | 0 (–) | 0 (–) | 85 (32) | 15 (4) | 0 (–) |
| 30–40 | 94 (11) | 6 (32) | 78 (37) | 15 (36) | 7 (69) | 100 (44) | 0 (–) | 0 (–) |
| 61–65 | | | | | | 100 (31)[a] | 0 (–) | 0 (–) |
| 85–88 | | | | | | 90 (41) | 10 (53) | 0 (–) |

[a]Suspected contamination of sample.

carbons in the Gulf of Maine core as measured by gas chromatography. The background hydrocarbon level for both areas appears to be 1–10 $\mu$g/g measured gas chromatographically.

Qualitatively, several differences can be seen between each station. The percentage of resolved hydrocarbons in each core section (Table 3, Fig. 1 and 2) shows a general increase in progressing away from Narragansett Bay and downcore at each station. The resolved hydrocarbon components are comprised primarily of normal, branched, and isoprenoid hydrocarbons, while the remaining unresolved complex mixture (UCM) is comprised of cycloparaffins plus aromatic and naptheno-aromatic hydrocarbons. We have previously noted the rapid decrease in the resolved and low molecular weight unresolved hydrocarbon components found in the Fields Point discharge and adjacent surface sediments in progressing downriver. At Station 3 in the lower Providence River (Fig. 1) some of the lower molecular weight unresolved components can still be seen. By mid-Narragansett Bay, all of these lower molecular weight components have been removed by chemical and/or microbial degradation or remobilization into the water column. The remaining hydrocarbons are primarily the higher molecular weight unresolved components. At the Rhode Island Sound station, these unresolved components still predominate in the surface sediments. In contrast, the near-surface sediments (1–2 cm) from the Gulf of Maine show a marked increase in the percentage of resolved components in the unbound fraction (5% resolved for Rhode Island Sound versus 25% for the Gulf of Maine). The percent resolved also increases with depth at each station (Table 3). The chromatograms clearly indicate the extent that anthropogenic hydrocarbons are distributed throughout Rhode Island coastal sediments.

The unresolved complex mixture, however, may not entirely represent petroleum hydrocarbon inputs. If the UCM were entirely due to petroleum inputs, we would expect that the UCM would completely disappear at a depth in the sediments equivalent to 75–100 yr B.P., the approximate age of the North American industrial revolution. The fact that the UCM is still significantly present at a depth of 85-88 cm in the Gulf of Maine core shows that this clearly is not the case. (If we assume that the sedimentation rate at the Gulf of Maine station is equal to or less than the sedimentation rate in nearby Buzzards Bay [Farrington et al. 1976], i.e. 2.9 mm/yr, the 85–88-cm depth would correspond to an approximate age of at least 300 yr.) It also shows that these complex hydrocarbons are not readily degraded and remain in the sediments for significant periods of time.

a conclusion that agrees with earlier work by Boehm and Quinn (1978), Farrington et al. (1977), and Van Vleet and Quinn (1977). Two likely sources for these unresolved hydrocarbons are natural pyrolytic sources such as forest fires, burning of coal and wood, and urban air fallout (Youngblood and Blumer 1975; Hase and Hites 1976; Farrington et al. 1977). Clearly in the older sections of the cores, pyrolytic sources are most probable.

The percentage of the total hydrocarbons in each section that are unbound, bound to humics, or bound to clays or kerogen (residual) is shown in Table 3. (Although it is probably inaccurate to refer to the hydrocarbons that are bound or closely associated with humics and/or clay minerals solely as "bound" hydrocarbons, for the sake of brevity, this terminology will be followed.) At each station, 78–100% of the hydrocarbons in each section are present in the unbound state and can be extracted directly with only the toluene–methanol mixture. In most cases, 90–100% of the hydrocarbons are unbound with only small amounts present in the remaining fractions. Hence, there appears to be no evidence of a diagenetic association between hydrocarbons and either humic substances or clay particles in our cores. In only a few cases were significant quantities of hydrocarbons (5–16%) associated with the humics and/or the residual fraction. This agrees with the earlier work of Farrington and Tripp (1975) and Van Vleet and Quinn (1977). In virtually all of the cases where significant association was found, the chromatograms of the bound hydrocarbons closely resembled the blank and may have been the result of contamination. In the analyses of unbound hydrocarbons from the Rhode Island Sound and Gulf of Maine, the concentration values were 5–60 times above the blank. In all of the results where bound hydrocarbons were detected in these two cores, the hydrocarbon values ranged only 0.3–1.5 times above the blank values. The difficulty in distinguishing these values from the blanks is another indication that they are relatively negligible. The unbound hydrocarbons from the Narragansett Bay core were 4–614 times above the blank values.

The resolved components in the sedimentary petroleum hydrocarbons appear to be degraded much more rapidly than the natural plant wax hydrocarbons. This may be largely due to the natural hydrocarbons being protected by the residual plant matrix, while the petroleum hydrocarbons are adsorbed on the surface of the sedimentary particles and are subject to much easier microbial and chemical degradation in the surface sediments. This relatively rapid breakdown of the resolved petroleum components is apparent in their rapid disappearance in the Providence River sediment cores (Van Vleet and Quinn 1977). The ability of the natural hydrocarbons to withstand microbial breakdown is witnessed in the emergence of the biogenic hydrocarbons $n\mathrm{C}_{25}$, $n\mathrm{C}_{27}$, $n\mathrm{C}_{29}$, and $n\mathrm{C}_{31}$ in the lower sections of these cores. These older sections of the core correspond to periods of lower petroleum input and hence a greater percentage of biogenic hydrocarbon input. The concentration of these biogenic hydrocarbons has been shown to remain relatively constant throughout the sediment core (Boehm and Quinn 1978; Farrington et al. 1977). The emergence of the biogenic hydrocarbons with depth can be followed by examining the odd–even preference (OEP) of *n*-alkanes (Scanlon and Smith 1970). The OEP of the Narragansett Bay core increased from approximately 1.0 at the surface to 2.6 at 30–40 cm, while the Rhode Island Sound core shows an increase in the OEP from 1.9 near the surface to 4.2 at 28–38 cm. In contrast, the near-surface sediments of the Gulf of Maine show an OEP of 3.0 that remains relatively constant throughout the core. The principal hydrocarbons responsible for the odd-carbon preference are $n\mathrm{C}_{25}$, $n\mathrm{C}_{27}$, $n\mathrm{C}_{29}$, and $n\mathrm{C}_{31}$, which are believed to be continentally derived from higher plants. The major *n*-alkanes present in these cores are $n\mathrm{C}_{29}$ and $n\mathrm{C}_{31}$.

The principal resolved component in the Narragansett Bay core (Kovat's retention index = 2153 on 8% FFAP; Fig. 1c) was previously identified as a cycloalkene of molecular weight 344, produced in the sediments either from biochemical synthesis by micro- or macroorganisms or by diagenetic alteration of a biogenic compound or suite of compounds (Boehm and Quinn 1978). This hydrocarbon was found to be the major resolved compound in the Rhode Island Sound core previously analyzed by Boehm and Quinn (1978). In addition, Boehm and Quinn (1978) found a second cycloalkene (mol wt = 348) which was lower in concentration than 344 but still made up a major portion of the resolved components. These components were not prominent peaks when we analyzed the same core. The two sets of analyses were performed approximately 6 mo apart during which the sediments were stored frozen at −20°C. The absence of the cycloalkenes in our analyses indicates that these components may have been oxidized during storage.

The exact source of the hydrocarbons in the Narragansett Bay and Rhode Island Sound sediments cannot be determined. As we noted earlier, the major single composite source appears to be the Fields Point treatment plant. The hydrocarbons discharged from the plant closely resemble a mixture of No. 2 fuel oil, No. 6 fuel oil, and used crankcase oil. These oils probably reach the treatment plant by several pathways including accidental discharge into sewer systems, disposal of used crankcase and heavy lubricating oils, oil washed from roads, and atmospheric deposition of hydrocarbons overland with subsequent washing into the sewage system. Most of the sedimentary hydrocarbons are probably a mixture of these petroleum products. According to Porricelli et al. (1971) up to 30% of the total petroleum input to the ocean may be from waste motor oil. While this estimate may be quite high, Tanacredi (1977) has also shown that used crankcase oil is a major contributor of oil to the coastal environments.

We have estimated an annual hydrocarbon input of 226 t from the Fields Point plant (Van Vleet and Quinn

1977). This plant serves a population of approximately 300 000 (H. P. Woodlock Jr., U.S. Army Corps of Engineers, personal communication) which gives an annual per capita usage of petroleum hydrocarbons of 0.000 753 t/(person·yr) entering from the Fields Point plant. This value can be compared with similar values from Norway — 0.001 45–0.005 85 (assuming one half of the oil and grease are due to petroleum hydrocarbons) (Foyn 1971), southern California — 0.002 92 (Storrs 1973), and Philadelphia — 0.0177 (Charter et al. 1973). Thus our estimate is apparently toward the lower end of the discharge range on a per capita basis. If we wish to extrapolate our value to a worldwide population of $4 \times 10^9$, it is necessary to make the following corrections and assumptions. First, we must correct for the fact that the United States (which constitutes only 5% of the world population) uses 30% of the total annual world oil production (Boyd et al. 1976). Second, we must account for the fact that only about 34% of the U.S. population lives in the coastal zone and discharges their municipal wastes into the coastal waters (NAS 1975). Third, we must assume that the worldwide geographical population trend is similar to the U.S. trend (e.g. 34% of the persons live in coastal areas). The extrapolation then results in an annual discharge of approximately 0.2 million t to the world's oceans from municipal wastes. This value is in good agreement with the previously estimated values (0.2–0.3 million t/yr) shown in Table 1 but may still be quite conservative due to the fact that our average per capita discharge from the Providence area is lower than the other U.S. estimates shown above. Our value of 0.2 million t/yr can account for approximately 3% of the total world input. If the per capita estimates from southern California or Philadelphia are more representative of the U.S. average, the municipal waste input can account for up to 42% of the total world input to the oceans. The actual value is probably between 5 and 10%. Based on a U.S. population of $2 \times 10^8$ persons, the Fields Point per capita discharge estimate of 0.000 753 t/(person·yr) gives a total annual U.S. discharge of approximately 150 000 t/yr. If only 34% of this is discharged by U.S. coastal communities, this amounts to approximately 51 000 t/yr discharged into U.S. coastal waters. This is slightly higher than our previous estimate of 31 000 t/yr (Van Vleet and Quinn 1977). If approximately 25 000 t enter U.S. coastal waters (versus 51 000 t for all U.S. waters) from direct spills each year (USCG 1975, 1976), it can be seen that municipal wastewater treatment plants can represent as significant a source of oil pollution to coastal waters as direct spills.

The results of this study indicate that municipal wastes may be a major contributor to oil pollution in U.S. coastal waters. These chronic petroleum inputs may represent as important a source of oil to coastal ecosystems as acute spills. The types of oil in these chronic inputs are most likely home heating oils, industrial heating and lubricating oils, and used crankcase oils. The present expense of building tertiary treatment facilities does not make this an economically attractive alternative. As suggested by Tanacredi (1977), perhaps a national waste oil recycling program could provide an economic incentive which would be useful as an inhibitor to coastal oil pollution as well as a significant energy conservation measure.

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# Preliminary Assessment of the Presence of Oil in the Ecosystem at Ekofisk After the Blowout, April 22–30, 1977[1]

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MACKIE, P. R., R. HARDY, AND K. J. WHITTLE. 1978. Preliminary assessment of the presence of oil in the ecosystem at Ekofisk after the blowout, April 22–30, 1977. J. Fish. Res. Board Can. 35: 544–551.

The marine environment in the Ekofisk area was assayed for the presence of oil components after the blowout on Platform Bravo was brought under control. Several methods of assay were used but the results were not always strictly comparable. Relatively high fluorescence values were observed in water samples in the vicinity of the platform. However, gas–liquid chromatography of these samples indicated that although some of the hydrocarbon fractions now resembled crude oil, none had increased markedly in concentration. The presence of oil could be detected in the biota and taste panels were able to identify an oily taint at low level in some fish caught near the platform. A second survey some 2 mo after the spill indicated that little, if any, oil from the blowout remained in the water column.

*Key words:* Ekofisk, petroleum, surface film, water, sediment, fish

MACKIE, P. R., R. HARDY, AND K. J. WHITTLE. 1978. Preliminary assessment of the presence of oil in the ecosystem at Ekofisk after the blowout, April 22–30, 1977. J. Fish. Res. Board Can. 35: 544–551.

Nous avons examiné l'environnement marin de la région d'Ekofisk en vue d'y déceler la présence de composants de pétrole une fois l'éruption maîtrisée sur la plate-forme de forage *Bravo*. Nous avons utilisé plusieurs méthodes d'analyse, mais les résultats ne sont pas toujours strictement comparables. On observe des valeurs de fluorescence relativement élevées dans les échantillons d'eau du voisinage de la plate-forme. Cependant, l'analyse par chromatographie gaz–liquide de ces échantillons indique que, bien que certaines des fractions hydrocarbures ressemblent maintenant à du pétrole brut, la concentration n'avait pas augmenté de façon notable dans aucun de ces échantillons. Il a été possible de déceler la présence de pétrole dans l'écosystème, et des groupes de dégustation ont pu identifier une légère saveur de pétrole dans certains poissons capturés à proximité de la plate-forme. Un deuxième relevé effectué quelque 2 mo après le déversement indique qu'il est resté très peu de pétrole, si tant est qu'il en est resté, dans la colonne d'eau à la suite de l'éruption.

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A mishap occurred in the course of engineering work on Platform Bravo in the Ekofisk oil field (Fig. 1) and a mixture of oil and gas was ejected from the well at a temperature of ~75–90°C to a height of ~50 m above sea level. The blowout continued for 7½ d from 22 to 30 April 1977. Estimates available at the time of writing suggest that between 20 and 30 000 tons of oil were released causing a slick of about 4000 km$^2$ to be formed mainly in the northeast quadrant from the rig (Institute of Marine Research 1977). Attempts to remove the oil from the sea surface met with little success due to gale force winds. Dispersants were used only in the area immediately adjacent to the platform to prevent extensive environmental damage.

The following is an account largely of chemical analytical observations made during two cruises, May 2–6 and July 5–12, 1977, by two of the British research vessels involved, the *Explorer* (May 2–6) and the *G.A. Reay* (July 5–12). The well had been capped by the time the first observations were made and sampling was concentrated on a small area northeast of the field (Fig. 1) where the oil slick was known to have been present. In this study an attempt was made to look in detail at the distribution of oil and its effects. The objective of the second cruise was to ascertain the amount and fate of any oil remaining in the area and more extensive coverage was possible (Fig. 2). Analyses from the second cruise are incomplete so this report will concentrate primarily on the observations made during the first cruise and will introduce only the preliminary results of the second cruise.

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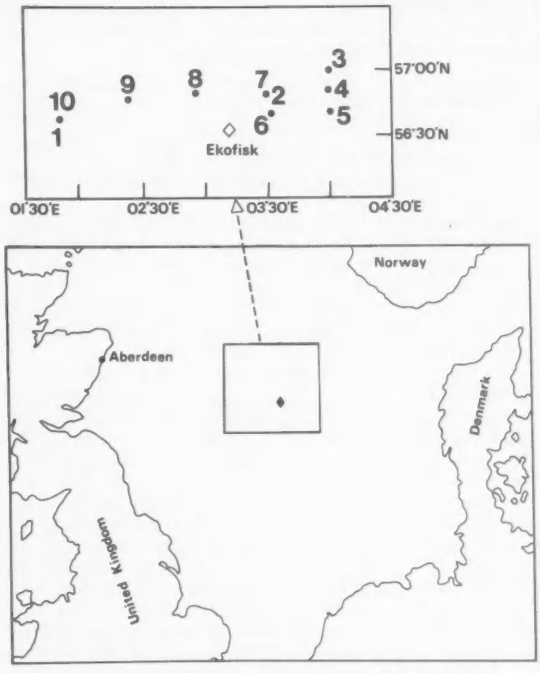


FIG. 1. Sampling stations in the area of the Ekofisk oil field, May 1977; ◆, Platform Bravo.

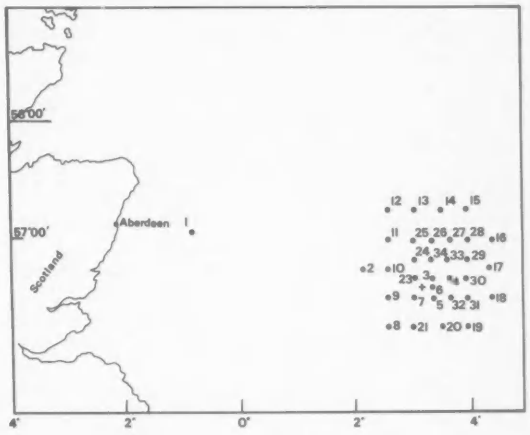


FIG. 2. Sampling stations in the area of the Ekofisk oil field, July 1977; +, Platform Bravo.

## Materials and Methods

### SAMPLES AND SAMPLING

The accurate determination of trace quantities of hydrocarbons in natural samples poses several problems in the sampling and extraction stages. Great care must be taken to purify chemicals and reagents and to clean thoroughly sampling equipment and glassware; otherwise the resultant contamination can swamp the hydrocarbons under examination. These problems and their solutions have been fully discussed elsewhere (Mackie et al. 1974, 1977). All glassware used in this work was thoroughly cleaned before use and interfering hydrocarbons removed from the solvents and reagents according to the methods of Mackie et al. (1974).

### FIRST CRUISE

The subsurface water was sampled according to Mackie et al. (1977). Water from 1 m deep was collected at each station in 2 × 2.5 L glass bottles held in a weighted frame. Immediately after the bottles were brought on board, 1 mL of squalane was added to one and 25 mL of chloroform to the other. After shaking, the bottles were stored at 1°C (the squalane bottle was inverted) until analyzed ~5–7 d later. Serial water samples were also collected from surface to bottom with a modified Menzel-Dazzler sampler. This is a 20-L capacity tubular Perspex container, 45 cm long, with internally mounted Perspex end plates. It was lowered to the desired depth with both ends open. The end plates were then closed by messenger and the sampler retrieved. A subsample was removed through the plastic stopcock in the main body of the sampler. Previous experience in which samples obtained at 20 m in this manner have been compared with those obtained by divers using 2.5-L bottles has shown that no contamination is incurred by the use of this sampler provided the first litre or so is discarded.

Aberdeen sediment traps (Payne and Davies 1977) were deployed at 2, 12, 23, and 33 m above bottom and recovered after 30 h. If oil was present on the surface waters above the traps they were carefully moved and brought through a surface that was free of visible floating oil.

Samples of fish (haddock, *Melanogrammus aeglefinus*; plaice, *Pleuronectes platessa*; gurnard, *Eutrigla gurnardus*; and lemon sole, *Microstomus kitt*) from bottom trawls at stations 1–4 and 6 were wrapped in aluminum foil and frozen whole as soon as possible after catching. Tissues for examination, muscle, liver, gonad (where reasonably developed), gill rakers, and gut (including stomach content), were excised in the laboratory while the fish were still partially frozen. This method of dissection minimizes changes caused by microbial and enzymatic activity and also losses due to drip and evaporation. The liver is particularly prone to degradation if ungutted fish are allowed to thaw completely.

### SECOND CRUISE

A similar sampling regime was employed on this cruise with the following differences. All the water samples were extracted immediately so as to minimize any changes due to sample storage. At stations that included surface film sampling, the 1-m depth water sample was collected from a small boat. This method has the advantage that the area surrounding the small boat is less likely to be contaminated. Aberdeen sediment traps were deployed at 2, 12, and 23 m above bottom. In addition, sediments were taken for chemical analyses by a van Veen grab and for benthic examination by a Smith–McIntyre grab (Smith and McIntyre 1954). The latter is spring loaded and can be weighted heavily. It gives a better depth profile than the simpler, pivoted van Veen which scraped the surface sediment. Unfortunately the Smith–McIntyre grab must be well lubricated for efficient operation and its use in chemical analytical work on

TABLE 1. Oil analysis by various methods of 1-m subsurface water samples near the Ekofisk oil field, May 2–6, 1977.

| Stn. no. | Fluorescence (μg crude oil equiv./L) | Volatiles (μg crude oil equiv./L) | $\sum C_{15}$–$C_{33}$ *n*-alkanes (μg/L) |
|---|---|---|---|
| 1 | 7.1 | 0.04 | 1.1 |
| 3 | 5.0 | 1.7 | 0.7 |
| 4 | 7.3 | — | 1.9 |
| 5 | 5.3 | 0.1 | 0.8 |
| 6 | 10.3 | — | 1.4 |
| 7 | 13.5 | 1.0 | 6.8 |
| 8(A) | 2.0 | 1.0 | 0.7 |
| (B) | 2.3 | 0.05 | 0.9 |
| 9 | 3.7 | <0.01 | 1.4 |
| 10(A) | 2.5 | 0.2 | 1.3 |
| (cf. No. 1) | | | |
| 10(B) | 3.3 | 0.05 | 0.5 |

oil is somewhat questionable. Cages containing 12 mussels were deployed on the bottom at 21 stations. We hoped that the mussels would be effective indicators of the hydrocarbon exposure during deployment. Some difficulty was experienced recovering the cages due to adverse weather and only eight cages were recovered. The surface film was sampled as previously described (Mackie et al. 1977). A piece of cleaned stainless steel mesh screen (200 mesh, 67 × 100 cm) was laid on the sea surface, rolled up, and placed in a glass extraction tube which was then stoppered. Care was taken to reduce handling of the screens to a minimum. Neuston tows of 1 km were performed using a plankton net (250 μm) with a 1-m-diameter mouth opening to collect tar balls, fish eggs, and larvae.

### EXTRACTION AND ISOLATION OF HYDROCARBONS

Purging techniques (Swinnerton and Linnenbom 1967) are used most commonly for the determination of volatile hydrocarbons in seawater. Unfortunately such equipment was not immediately available to us and we decided to attempt to extract the volatile components present in the seawater with squalane which was gas chromatographically free of volatile impurities. Direct injection of the squalane onto the gas chromatograph would indicate the presence of low molecular weight compounds without interference from solvent. The volatiles were not reexamined during the second voyage.

The unfiltered water was extracted twice with 50-mL aliquots of chloroform. The pooled, dried (anhydrous $Na_2SO_4$) extracts were quantitatively transferred to 5 mL of pentane and 1 mL of the pentane removed for spectral analysis. The remaining 4 mL were fractionated into alkane and polynuclear aromatic hydrocarbon (PNAH) components as previously described (Mackie et al. 1974).

The surface film was extracted with chloroform–methanol as outlined in Mackie et al. (1977) and the chloroform treated as above. The particulate material from the sediment traps was extracted monophasically with chloroform–methanol and again treated as above.

The fish tissues were extracted according to Bligh and Dyer (1959) and the fractions isolated according to Mackie et al. (1974). A portion of the muscle was steamed for 30 min in a closed casserole for assessment of oily taints by a taste panel (Howgate et al. 1977). Squalane (1 μg) was added as an internal standard to all samples (water, surface film, sediment, and tissues) prior to extraction.

### UV AND FLUORESCENCE SPECTROSCOPY AND GAS CHROMATOGRAPHY

The pentane solvent from 20% of the water extract (1 mL) was replaced by 2 mL isooctane and the fluorescence spectra recorded on an Aminco-Bowman SPF (spectrophotofluorometer) with excitation at 310 nm; quantitation was performed on the emission reading at 360 nm (IGOSS 1976) and the crude oil determined by reference to a calibration curve using whole Ekofisk crude. The same solution was used to obtain the UV and visible spectrum on a Cecil Model 505 spectrophotometer. Both these instruments were used shipboard during the second cruise.

Two glass capillary columns were used for the analysis of the volatile material. The first, a 40-m squalane-coated column, operated at 28°C was used for gaseous compounds; hydrocarbons in the range $C_{10}$–$C_{12}$ were determined on a 30-m column coated with OV-101 and operated at 104°C. Higher molecular weight alkanes ($C_{15}$–$C_{28}$) were analyzed on the same OV-101 column programmed from 90 to 280°C at 3°C/min. These columns were coupled (McGill et al. 1977) to Pye 104 gas chromatographs with injection parts modified by the addition of SGE glass-lined splitters. The PNAH were separated on a 15-m SE-52 glass capillary installed in a Carlo Erba 2150 gas chromatograph and programmed from 100 to 200°C at 2°C/min. In all cases the carrier gas was oxygen-free nitrogen with a flow rate of 0.75 mL/min and a split ratio of 15:1. All the glass capillary columns were made at the Torry Research Station.

## Results and Discussion

### FIRST CRUISE MAY 2–6, 1977

The results of the chemical analyses of the subsurface waters are summarized in Table 1. The fluorescence estimations of crude oil concentrations show that with the exception of Station 1, there was less crude oil present to the west of Ekofisk than to the east. This distribution is not confirmed by the other analytical results, although the Station 7 sample contained the highest concentrations of fluorescing material and long-chain *n*-alkanes, as well as a relatively high concentration of volatile components. A more detailed examination of the analytical data shows a number of anomalies. Thus, only the fluorescence spectra obtained from samples at Stations 7 and 3 approximate that of crude oil, and some spectra such as those from Stations 5 and 6 show marked differences. No doubt localized weathering of any crude oil present could account for some of these differences. However, using similar extraction techniques we have obtained substances from extracts of cultured phytoplankton that give fluorescence spectra similar to those obtained in this study.

The results of analyses carried out on the waters obtained from greater depths are given in Table 2. Although the concentration of fluorescent components are lower than those found in water samples taken at 1 m deep, with the exception of Station 6, the deeper

TABLE 2. Oil analysis by fluorescence of water from various depths near the Ekofisk oil field, May 2–6, 1977.

| Stn. no. | Depth (m) | Concn (μg crude oil equiv./L) |
|---|---|---|
| 1 | 5 | |
| | 15 | |
| | 25 | 0.8 or less |
| | 45 | |
| | 65 | |
| 3 | 5 | |
| | 15 | |
| | 25 | 0.8 or less |
| | 35 | |
| | 45 | |
| 4 | 5 | 3.6 |
| | 15 | 3.3 |
| | 25 | 4.3 |
| | 45 | 3.4 |
| 6 | 5 | 13.0 |
| | 15 | 13.5 |
| | 25 | 15.2 |
| | 35 | 13.4 |
| | 45 | 2.0 |

waters at Stations 4 and 6 show consistently higher concentrations than 1 and 3. If these higher concentrations are indeed due to oil components, then the results indicate that oil had dispersed throughout the water column to the east of the platform. Hydrographic measurements made during this cruise indicated that the waters in the survey area were well mixed with depth and so vertical dispersion would be expected.

Low-temperature analyses of the squalane extracts indicate that all the water samples had concentrations of low molecular weight hydrocarbons ($C_1$–$C_6$) of $<0.01$ μg/L, assuming complete dissolution in the squalane. An analysis at a higher temperature indicates that components within the boiling range of $C_1$–$C_{12}$ *n*-alkanes were present in the water. Comparison of the peak areas so obtained with those of standard solutions of Ekofisk oil yielded the figures quoted in Table 1. The chromatograms of the squalane extracts show little specific detail and thus, like the fluorescence spectra, are not necessarily indicative of crude-oil components.

In the past a number of *n*-alkane measurements have been made on North Sea waters and average concentrations ($\Sigma C_{15}$–$C_{33}$) ranging from 0.166 μg/L ($\sigma = 0.073$) (Mackie et al. 1976) to 4.5 μg/L ($\sigma = 1.6$) (Hardy et al. 1977) have been recorded. Therefore, the values given in Table 1, although on the high side when compared with the most recent analyses, are not outside past analytical experience. In a number of samples the composition of the *n*-alkanes (see Table 3) was different from that previously observed in North Sea waters. This is shown graphically in Fig. 3, where the *n*-alkane distribution of the 1-m water from Station 7 is depicted with mean distribution obtained from previous United Kingdom surveys (Hardy et al. 1977). The subsurface water from other stations, namely 4 and 6, possessed *n*-alkane composition similar to that of Station 7 and are very reminiscent of weathered crude oils. Water samples from these three stations together with

TABLE 3. Alkane analysis of 1-m subsurface water from near the Ekofisk oil field, May 2–6, 1977.

| Carbon no. | Station no. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 3 | 4 | 5 | 6 | 7 | 8(A) | 8(B) | 9 | 10(A) | 10(B) |
| 15 | 0.059 | 0.049 | 0.153 | 0.045 | 0.068 | 0.367 | 0.023 | 0.033 | 0.076 | 0.081 | 0.006 |
| 16 | 0.052 | 0.044 | 0.142 | 0.042 | 0.085 | 0.514 | 0.025 | 0.037 | 0.076 | 0.051 | 0.006 |
| 17 | 0.070 | 0.059 | 0.155 | 0.054 | 0.166 | 0.683 | 0.025 | 0.044 | 0.093 | 0.071 | 0.014 |
| 18 | 0.058 | 0.056 | 0.170 | 0.047 | 0.110 | 0.736 | 0.020 | 0.039 | 0.085 | 0.040 | 0.017 |
| 19 | 0.046 | 0.050 | 0.177 | 0.044 | 0.119 | 0.720 | 0.020 | 0.043 | 0.076 | 0.037 | 0.009 |
| 20 | 0.035 | 0.038 | 0.172 | 0.043 | 0.105 | 0.625 | 0.023 | 0.035 | 0.058 | 0.032 | 0.014 |
| 21 | 0.034 | 0.035 | 0.151 | 0.037 | 0.096 | 0.494 | 0.029 | 0.035 | 0.050 | 0.027 | 0.023 |
| 22 | 0.048 | 0.035 | 0.125 | 0.037 | 0.080 | 0.404 | 0.034 | 0.027 | 0.068 | 0.029 | 0.023 |
| 23 | 0.053 | 0.031 | 0.123 | 0.032 | 0.074 | 0.340 | 0.032 | 0.035 | 0.053 | 0.029 | 0.020 |
| 24 | 0.070 | 0.029 | 0.082 | 0.034 | 0.064 | 0.282 | 0.032 | 0.048 | 0.097 | 0.047 | 0.023 |
| 25 | 0.094 | 0.041 | 0.078 | 0.037 | 0.077 | 0.262 | 0.054 | 0.062 | 0.093 | 0.064 | 0.032 |
| 26 | 0.096 | 0.041 | 0.082 | 0.040 | 0.069 | 0.232 | 0.070 | 0.078 | 0.102 | 0.081 | 0.036 |
| 27 | 0.088 | 0.038 | 0.069 | 0.052 | 0.060 | 0.225 | 0.065 | 0.078 | 0.108 | 0.094 | 0.049 |
| 28 | 0.079 | 0.038 | 0.060 | 0.062 | 0.057 | 0.205 | 0.063 | 0.078 | 0.100 | 0.121 | 0.043 |
| 29 | 0.085 | 0.038 | 0.060 | 0.058 | 0.047 | 0.182 | 0.051 | 0.073 | 0.079 | 0.101 | 0.043 |
| 30 | 0.064 | 0.035 | 0.047 | 0.054 | 0.041 | 0.148 | 0.042 | 0.060 | 0.071 | 0.084 | 0.035 |
| 31 | 0.037 | 0.032 | 0.043 | 0.052 | 0.038 | 0.135 | 0.040 | 0.049 | 0.063 | 0.067 | 0.030 |
| 32 | 0.037 | 0.027 | 0.034 | 0.039 | 0.027 | 0.118 | 0.026 | 0.037 | 0.055 | 0.182 | 0.023 |
| 33 | 0.024 | 0.015 | 0.026 | 0.034 | 0.019 | 0.104 | 0.018 | 0.028 | 0.050 | 0.047 | 0.020 |
| $\sum_{33}^{15}$ | 1.129 | 0.731 | 1.949 | 0.843 | 1.402 | 6.776 | 0.692 | 0.919 | 1.453 | 1.285 | 0.466 |
| Pristane | 1.252 | 0.834 | 1.214 | 1.073 | 0.535 | 1.392 | 0.487 | 0.789 | 0.959 | 1.293 | 0.503 |
| Phytane | 0.041 | 0.035 | 0.078 | 0.032 | 0.060 | 0.289 | 0.018 | 0.022 | 0.050 | 0.034 | 0.013 |

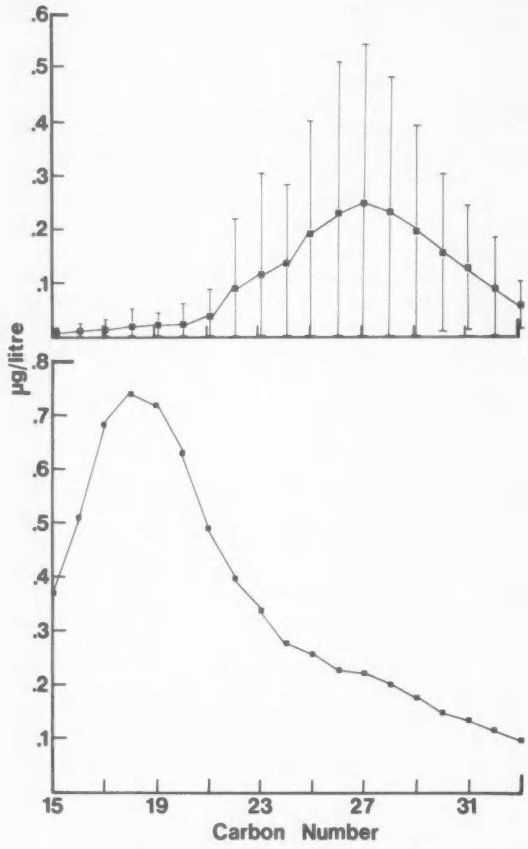


FIG. 3. *n*-Alkane distributions with respect to carbon number in subsurface waters. *Upper graph*: mean values obtained from the U.K. coastal survey including standard deviations (from Hardy et al. 1977). *Lower graph*: Station 7 (May 1977). Lines joining the carbon numbers are for visual convenience.

those of Station 1 also contained other hydrocarbon components very similar to those present in Ekofisk oil especially in the $C_{16}$–$C_{19}$ region of the chromatogram. This is shown in Fig. 4A and B. Pristane and phytane are present in much higher concentrations compared with previous analyses, and being components of crude oil, it is tempting to assume that they are derived from it. However, apart from Station 7, the ratios pristane/phytane, pristane/$nC_{17}$, and phytane/$nC_{18}$ are not consistent with those of our sample of the crude. On this basis, it is questionable whether their presence in samples other than Station 7 arises largely from the crude oil unless the differences are due to varying stages of weathering.

Again with the exception of Station 7, GLC analysis showed that the water samples contained only very small quantities of aromatic hydrocarbons similar to those found in the Ekofisk oil. The qualitative, though not quantitative, distribution of aromatic hydrocarbons in the sample at Station 7 was similar to that of Ekofisk crude. These results suggest that the high fluorescence values of the samples, apart from that of Station 7, may not arise from the presence of oil-derived aromatic hydrocarbons.

Summing up the water analyses, it seems that chemical components similar to those found in oil were present especially in areas to the east of the platform. Nevertheless, the composition of the samples in many instances indicates either that the oil had undergone marked compositional changes or that components not derived from the oil were giving rise to spuriously high values.

Although considerable quantities of gas were lost no large concentrations of low molecular weight material were found. Based on the higher oil concentrations measured in these analyses and the water volume in the sampling area around the platform, we estimate that ~10% of the escaped oil can be accounted for in the water column. This suggests either that dispersion occurred in a much larger volume of water or that considerable quantities of oil were lost to the atmosphere or to the sediment.

The detailed analysis of the sediment trap material has not yet been completed but the fluorescence spectra suggest that this material contains oil components (Table 4). Whether or not this would account for substantial quantities of oil is not known; certainly the lack of any sizeable surface film of oil in the sampling area indicates that residence time at the surface was short.

Samples of haddock, plaice, gurnard, and lemon sole were obtained at Stations 1, 2, 3, 4, and 6 and the hydrocarbon content and composition of the various tissues determined. No positive identification of Ekofisk oil components was made in the majority of the samples. In six of the samples, namely the gut and stomach contents of mackerel from Station 1, haddock from Stations 2, 3, 4, and 6, and plaice from Station 6, there were unmistakable signs of Ekofisk oil although alkane levels were not elevated. Figure 4C shows part of the alkane chromatogram of haddock gut from Station 6, and its similarity with the Ekofisk crude oil (Fig. 4A) is apparent.

The taste-panel results are shown in Table 5. These show that in all cases taints were detected by a few panel members but only by the majority of the panel in the case of haddock at Stations 2 and 4. No oil-derived hydrocarbons could be found in the muscle, perhaps confirming the data of Howgate et al. 1977, who found that some of the major flavor components in crude oil are not hydrocarbons. It has been noted in the past that the ease with which fish acquire various oil components depends on the nature of the oil and fish (Hardy et al. 1974). Thus the fish caught in the vicinity of the blowout may have been tainted with oil even though hydrocarbon components are absent in the majority of their tissues. The fish that possessed oil components feed

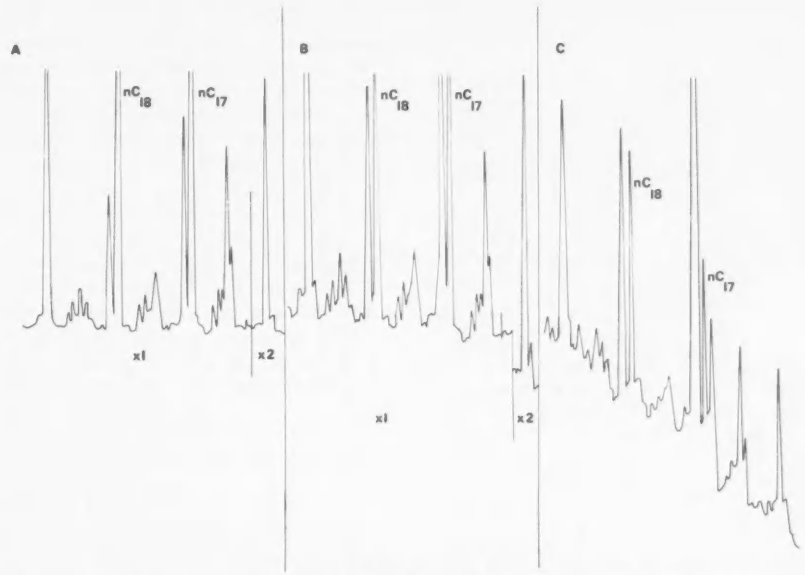


FIG. 4. Partial chromatograms of the alkane fraction of (A) Ekofisk crude oil, (B) subsurface water from Station 7, and (C) gut content of haddock from Station 6. (Both sampled in May.) Operating conditions are given in the text.

TABLE 4. Amount and oil content of sedimenting material at Station 2(7), May 1977.

| Metres above bottom | Dry wt (mg/m² per d) | Crude oil content (equiv. μg/mg) |
|---|---|---|
| 2 | 365.4 | 1.0 |
| 12 | 69.1 | 4.2 |
| 23 | 23.7 | 10.4 |
| 33 | 28.2 | 21.8 |
| Control (Fladen ground) | | |
| 12 | — | 0.8 |

at or near the bottom, and considering that the greatest concentration of oil was found in the stomach it is possible that oil was transported to the bottom and picked up by the fish there rather than from the water column. The sediment trap analyses indicate that some oil was sedimenting out and thus reaching the bottom. It is therefore quite feasible that bottom feeding fish were able to ingest oil attached to particulates. The mackerel, with a stomach content of plankton, presumably acquired oil from the plankton which have the ability to accumulate hydrocarbons (Harris et al. 1977).

One noticeable feature of these analyses is the apparent presence of oil in many of the samples from Station 1. The only inference that can be drawn concerning the water column is that there had been an oil spill at this site unconnected with the Bravo blowout. Support for this hypothesis is provided by the results from Station 10 (identical site to that of Station 1 but sampled 2 d later)

TABLE 5. Taste panel assessment of taint from fish samples caught in May 1977. The numerator is the number of people from a panel who stated that the flesh had a definite petroleum-type oily taint; the denominator is the number of people in the panel.

| Stn. no. | Haddock | Plaice | Gurnard | Lemon Sole |
|---|---|---|---|---|
| 1 | $\frac{2}{6}$ | $\frac{2}{7}$ | $\frac{2}{6}$ | — |
| 2 | $\frac{4}{6}$ | $\frac{1}{7}$ | $\frac{3}{6}$ | $\frac{2}{7}$ |
| 3 | $\frac{1}{6}$ | $\frac{3}{7}$ | — | $\frac{3}{7}$ |
| 4 | $\frac{5}{6}$ | $\frac{2}{7}$ | $\frac{3}{6}$ | $\frac{1}{7}$ |
| 6 | $\frac{1}{6}$ | $\frac{1}{7}$ | $\frac{1}{7}$ | $\frac{3}{7}$ |

in which little oil could be detected. This area is subjected to a large number of relatively minor spills and during the second cruise some apparently fresh slicks were observed.

## SECOND CRUISE JULY 5–12, 1977

Temperature measurements indicated that the water column around Ekofisk was well mixed during the first cruise; in the intervening period before the second cruise stratification had taken place.

At the time of writing only the UV and fluorimetric analyses on samples obtained from this cruise have

TABLE 6. Surface tar ball and oil analysis of water from various depths near Ekofisk, July 5–12, 1977. (Trace (tr) <0.2.)

| Stn. no. | Surface film ($\mu$g crude oil equiv./m$^2$) | Tar balls (mg/m$^2$) | Subsurface water $\mu$g crude oil equiv./L | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 m | 10 m | 30 m | 50 m | 5 m above bottom |
| 1 | | 0.03 | 4.0 | | | | |
| 2 | | 0.01 | 1.0 | | | | |
| 3 | 88.0 | | 1.0 | 7.5 | 3.1 | | 3.1 (50 m) |
| 4 | | | 3.0 | | | | |
| 5 | | | 1.0 | | | | |
| 6 | | | 1.6 | | | | |
| 7 | 27.0 | 1.07 | 1.8 | 1.75 | 1.7 | 1.8 | 1.8 (65 m) |
| 8 | 16.6 | <0.01 | 0.8 | 0.8 | 0.7 | 0.7 | 0.8 (70 m) |
| 9 | | <0.01 | 0.8 | | | | |
| 10 | 2.2 | 0.39 | 0.8 | | | | |
| 11 | | | 0.5 | | | | |
| 12 | 30.0 | 0.09 | 0.5 | 0.7 | 0.5 | 0.5 | 0.5 (70 m) |
| 13 | | | 0.7 | | | | |
| 14 | 32.2 | 0.02 | 0.8 | | | | |
| 15 | | | 1.0 | | | | |
| 16 | | | 0.8 | | | | |
| 17 | 22.6 | <0.01 | 0.9 | | | | |
| 18 | | | 0.8 | | | | |
| 19 | | | 0.9 | | | | |
| 20 | 25.1 | 8.74 | 0.8 | | | | |
| 21 | | | 0.8 | | | | |
| 22 (cf. no. 3) | 264.4 | 0.72 | | | | | |
| 23 | | | 3.1 | | | | |
| 24 | | | 0.8 | | | | |
| 25 | 15.6 | 0.36 | 1.0 | 0.9 | 0.9 | | tr (50 m) |
| 26 | | | <0.2 | | | | |
| 27 | | | <0.2 | | | | |
| 28 | 18.8 | <0.01 | 0.8 | | | | |
| 29 | 17.9 | | 0.7 | | | | |
| 30 | 18.8 | | <0.2 | | | | |
| 31 | | | 0.6 | | | | |
| 32 | 23.7 | 0.01 | <0.2 | | | | |
| 33 | | | <0.2 | | | | |
| 34 | | 0.10 | <0.2 | | | | |
| 46 | 34.2 | | | | | | |

been completed. Ekofisk oil exhibits a typical oil spectrum (Levy 1972) with maximum absorption at 228 and 244 nm and a high level of general absorption throughout the rest of the UV and visible ranges. This characteristic absorption pattern was observed in only one of the extracts analyzed. In many instances an absorption peak was observed at 228 nm but with the exception of the surface film from Station 22 no absorption peak was noted at 244 nm. Apart from this there were two other outstanding features in the spectra: all the extracts from surface films were alike as were those from the water but the two sets differed from one another. Thus the surface film extracts exhibited maximum absorption at 222, 276, and occasionally 546 and 674 nm, the strongest absorption being at 222 nm (range 0.9–1.9 optical density units). The strongest absorption of the water extracts occurred most usually at 210 nm (range 0.075 to >0.25), sometimes at 228; weak absorption peaks occurred at 426 and 440 nm and others, usually still weaker, at 476, 544–46, and 668–74 nm. The intensities of the spectra varied with site and depth but not in any simple manner or one that could be correlated with the spillage. It is interesting that phytoplanktonic extracts gave UV spectra fairly similar to those of the waters. The possibility arises that the organic extractable components of the biota are primarily responsible for the spectral characteristics of the samples.

The fluorescence estimations of oil concentrations are given in Table 6 and those made for the subsurface water at 1 m may be compared with those of the first cruise (Table 1). Almost without exception the estimates are lower, indicating the loss of oil from this layer of the water. Once again the spectra are not identical with crude oil, and as in the first cruise, the estimates given in the table should not be accepted without some reservations. Apart from Station 3, components exhibiting fluorescence spectra tended not to vary in concentration throughout the water column. Station 3 had a relatively high surface-film concentration which was even greater when the ship returned later (Station 22). At

this time a thin oil slick could be seen; the surface film at this station was the only one that gave a UV absorption spectrum similar to that of crude oil. No simple correlation could be found between the intensities of the UV absorption spectra and the fluorescence spectra. Results from the neuston tows indicate that the incidence of tar balls was much greater (Table 6) than the incidence of those observed in any of our previous, albeit limited, samplings which were of the same order as those observed by Smith (1976). The tar balls on this cruise ranged from pinhead size to one weighing over 4 g.

Except at Station 9 no abnormalities were noted in the fish eggs and larvae caught by the neuston net. In this sample several dead eggs were found that appeared to have oily encrustations on the outer membrane extending into the yolk.

A complete absence of oily taints from the fish (cod, haddock, and plaice) tasted aboard ship was noted by a taste panel.

From the results we have obtained to date it would seem that although a very large quantity of oil was spilt in the Bravo blowout, high concentrations were not found in the water immediately after the flow was halted. It follows that the redistributive processes must have been rapid. Because of the volatility of the oil it is most probable that evaporation was the most important of these, although some oil appeared to sediment to the bottom. After a further 2 mo, positive identification of oil in the water column could not be made except at one position (Station 3) and it thus seems that only very small amounts of oil remained. The results of the various methods used were not always in agreement when expressed in terms of oil concentration. This could be because the various components of the oil had differing fates or because other components present in the water were interfering with the determinations. Although experimental studies in the laboratory indicated that the water near the platform was relatively innocuous to marine biota, taste panel and analytical studies showed that oil components had been acquired by fish. The taste panel results, although indicating that any oily taints cleared over a short period, are impossible to interpret unequivocally because it is unlikely that the fish population sampled was static. The area around the platform does not support a major fishery, so from this point of view the oil contamination is not serious. It is, however, a major spawning ground for mackerel and although the blowout preceded the spawning season by a few weeks, thus avoiding mortality, we hope that any more subtle effects will be insignificant.

## Acknowledgments

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# Weathering Characteristics of Petroleum Hydrocarbons Deposited in Fine Clay Marine Sediments, Searsport, Maine[1]

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Further examination of the 1971 spill site in Long Cove, Searsport, Maine, has been carried out during 1976, and has led to the following observations: 1. Petroleum residues isolated from this spill give the appearance of weathering particularly slowly in the cold anoxic sediments of Long Cove. 2. Leaching from contaminated terrestial sediments onto portions of the intertidal zone on the west shore of the Cove while continuing in trace concentrations does not appear to be making a major contribution to the maintenance of a nearly steady state pollution problem found in several areas of the Cove. 3. On the average, the spill area currently appears to contain roughly 20% less material than in 1971. At a number of sites, however, there appears to have been either little or no decline in gross hydrocarbon concentrations, and essentially no weathering of the aliphatic portions of the petroleum residues. 4. One of the principal factors to influence the character of petroleum residues isolated 5 yr postspill appears to have been the weathering of the hydrocarbons during transport to the site of sediment penetration from upland locations. Thus, the current compound distribution was determined early in the spill period. 5. Repopulation of the Cove by *M. arenaria*, which is proceeding very slowly, correlates directly with the gross concentration variations of petroleum hydrocarbons.

*Key words:* petroleum, gas chromatography, weathering, clay sediments, Maine

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Le site du déversement de pétrole de 1971 dans l'anse Long, Searsport, Maine, fut réexaminé en 1976 et on a fait les observations suivantes : 1. Les résidus de pétrole isolés de ce déversement donnent des indications que l'altération à l'air fut particulièrement lente dans les sédiments anoxiques froids de l'anse Long. 2. Le lessivage des sédiments terrestres contaminés dans des portions de la zone intertidale sur la rive occidentale de l'anse, bien que se poursuivant à des concentrations de traces, ne semble pas contribuer de façon importante au maintien d'un problème de pollution presque permanente observée dans plusieurs parties de l'anse. 3. Dans le moment, le déversement semble contenir en moyenne environ 20% moins de matériel qu'en 1971. A plusieurs endroits cependant, il semble y avoir eu diminution faible ou nulle des concentrations brutes d'hydrocarbures et essentiellement absence d'altération des portions aliphatiques des résidus de pétrole. 4. Un des principaux facteurs à avoir influencé le caractère des résidus de pétrole isolés 5 ans après le déversement semble être l'altération à l'air des hydrocarbures durant le passage depuis les hautes terres jusqu'au site de pénétration dans le sédiment. La répartition actuelle des composés fut donc déterminée tôt durant la période du déversement. 5. La recolonisation de l'anse par *M. arenaria*, qui se poursuit à un rythme très lent, est en corrélation directe avec les variations de concentration brute d'hydrocarbures de pétrole.

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DURING early March 1971 a United States Air Force Fuel Depot located adjacent to the west shore of Long Cove, Searsport, Maine, suffered a pipeline rupture. The escaping petroleum, later identified as a mixture of JP4-jet fuel and No. 2 heating oil, eventually reached the intertidal zone of the Cove on March 16, 1971. The initial volume of the spill was at least 14 t based on recovered material and the actual spill very likely was considerably above the minimum estimate. The loss of resident *Mya arenaria* was large and it has been estimated that approximately 135 t of a standing crop of 157 t were destroyed by August 1974 (Dow and Hurst 1975). Four collections of Cove sediment samples were made from March 1971 through September 1972. Analysis of these samples indicated substantial contamination of the Cove with light to medium refined hydrocarbons, with the highest concentrations centered on the west shore (Mayo et al. 1972). During 1976 a follow-up study of the region was sponsored by the Maine Department of Environmental Protection (Gilfillan et al. 1977). Sediment and tissue samples were collected from a large number of areas including several stations close to the original sediment sampling sites. This paper reports the analysis of those recently collected sediment samples in which it was possible to make a comparison with hydrocarbon data collected in the earlier investigation. In addition, an assessment of the current state of recovery of Long Cove from the 1971 oil spill will be considered. This evaluation is based on the interaction of *Mya* populations with petroleum residues and employs these animals as an index of the return of the Cove to a normal prespill state.

## Materials and Methods

Sediment samples were analyzed for hydrocarbon residues using the procedure given below. This methodology has been shown in this laboratory to give 85% or better recovery of a No. 2 fuel oil spike and ±10% or better reproducibility.

Sediment samples were collected in the field in ½-pint mason jars with aluminum foil-lined caps. Both the jars and the foil liners were rinsed with methanol and then with *n*-pentane prior to sample collection. The upper 15 cm of sediment at each station was taken using a coring device. The data, then, represent an integration of the hydrocarbons present in the upper 15-cm stratum at each site.

All solvents used in the extraction and work-up procedure were glass distilled and checked for purity by gas-chromatographic analysis before use. Special high purity *n*-pentane was obtained from Phillips Petroleum, Inc. and the other solvents were reagent grade. All extraction thimbles and drying agents were extracted for 24 h in a Soxhlet extractor using *n*-pentane as a solvent to remove trace hydrocarbon contaminants.

Each sediment sample was air dried at room temperature and 100 grams of dried sediment was weighed out in an extraction thimble. The sample was extracted for 24 h with *n*-pentane followed by extraction for 24 h with 60% *n*-pentane–40% benzene. Both the pentane extract and the pentane–benzene extract were concentrated to a volume of 5 mL using a rotary evaporator. The concentrated pentane fraction was transferred through a cotton plug to a 1 × 50-cm liquid chromatography column containing the following materials: (top to bottom) anhydrous $Na_2SO_4$/activated copper/alumina/silica. In each column, 3.4 g of activity 1 silica gel, 6.6 g activity 1 alumina, 1 mL of activated copper powder, and 3 g of anhydrous $Na_2SO_4$ were used. The use of a multilayered column accomplished dehydration, sulfur removal, and liquid chromatography in one step and thus eliminated several transfers had the steps been done separately (Mayo et al. 1978). The pentane fraction was allowed to reside in the $Na_2SO_4$ part of the column for 1 h to ensure drying and then eluted with 3 column volumes of *n*-pentane to yield the "aliphatic fraction" of hydrocarbons present in the sediment sample. The concentrated pentane–benzene sediment extract was then applied to the column as above and eluted with 3 column volumes of 50% benzene–pentane to yield the "aromatic fraction" of the hydrocarbons present in the sediment sample. Both the aliphatic fraction and the aromatic fraction were concentrated to a residue under nitrogen to a constant weight. The residue weight (±0.01 mg) was then obtained for each fraction.

The aliphatic fraction from each sediment sample was analyzed by gas chromatography using a Perkin-Elmer model 990 gas chromatograph with dual flame ionization detectors, an MS-41 solid sampling injection system, and helium as the carrier gas. An all-glass 27-m SE-30 SCOT column was used. The operating conditions were the same for all samples analyzed. On injection of the sample in $CS_2$, an initial temperature of 80°C was maintained for 1 min. The temperature was then programmed at 4 deg/min to a final temperature of 300°C which was maintained for 12 min.

## Results and Discussion

A number of the 1976 sediment collection sites were located as near to the 1971 sites as could be determined from the data available to establish any possible correlations with the 1971–72 data. The aliphatic fraction of the petroleum residues of the earlier samples had been analyzed by gas-chromatographic analysis employing 1.8-m dual packed columns with Apiezon-L as the stationary phase (Mayo et al. 1972). A number of the residues collected from these sites, however, were subsequently rerun on a SCOT 15-m stainless steel column using OV-1 as the stationary phase (Mayo et al. 1975). These latter samples were of considerable interest for comparison as the current data was obtained with a 27-m, glass SCOT column utilizing SE-30 as the stationary phase. While the isolation techniques differed slightly between the 1971 and the 1976 study, these changes reflect primarily increased efficiency of transfer operations and more accurate quantitative analyses. These changes have not reflected any measurable change in compound distribution in the isolated residues, the latter observation being central to the arguments to follow.

Sites where a detailed analysis was carried out are identified in Fig. 1. In nearly all cases, the gross hydrocarbon concentrations at these sites in 1976 were similar or slightly depressed relative to 1971 values (see Table 1). Furthermore, the compound distributions within the isolated mixtures (established by gas chromatography) were virtually identical to those values ob-

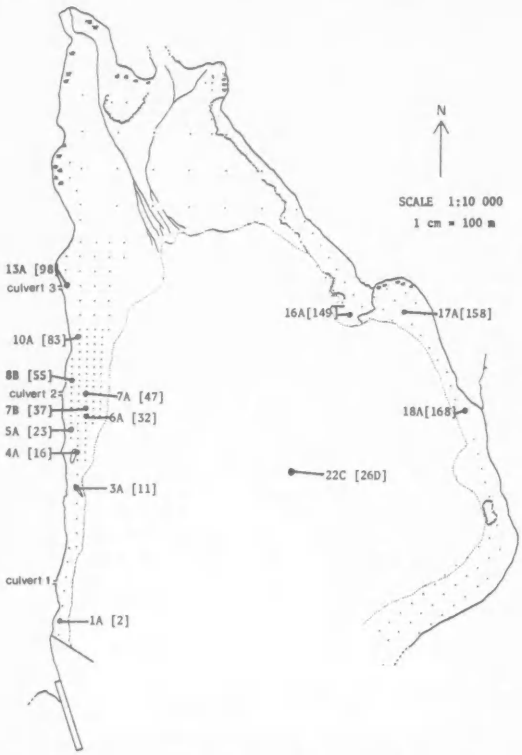


FIG. 1. Location of stations in Long Cove employed in intrasite comparisons in the text between the 1971–72 study and the 1976 study. 1976 station numbers are enclosed in brackets.

TABLE 1. Comparison of total hydrocarbon residues (ppm) isolated from those stations in Long Cove where 1971 and 1976 sites were coincident.

| Site no. | 1971 gross wt (ppm) | 1976 gross wt (ppm) |
|---|---|---|
| 1A [2][a] | 137 | 175 |
| 3A [11] | 254 | 250 |
| 4A [16] | 212 | 133 |
| 5A [23] | 143 | 78 |
| 6A [32] | 88 | 162 |
| 7A [47] | 74 | 153 |
| 7B [37] | 210 | 145 |
| 8B [55] | 82 | 6 |
| 10A [83] | 53 | 39 |
| 13A [98] | 49 | 12 |
| 16A [149] | 7 | 7 |
| 17A [158] | 158 | 8 |
| 18A [168] | 82 | 31 |
| 22C [26D] | 200 | 180 |
| Avg value | 125 | 99 |

[a]Corresponding 1976 station number given in brackets following 1971 identifying registration.

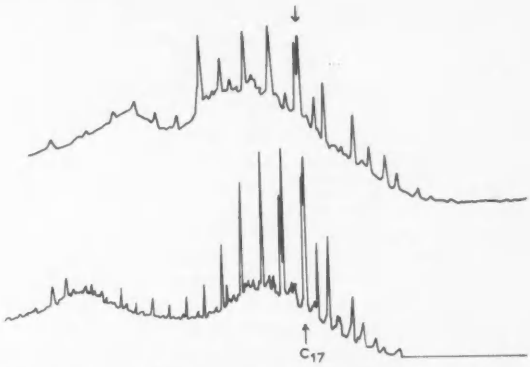


FIG. 2. (*top*) Gas chromatogram of sediment sample from station 5A (1971); Apiezon-L packed column. (*bottom*) Gas chromatogram of sediment sample from station 23 (1976); SE-30 SCOT column. Retention time increases R to L.

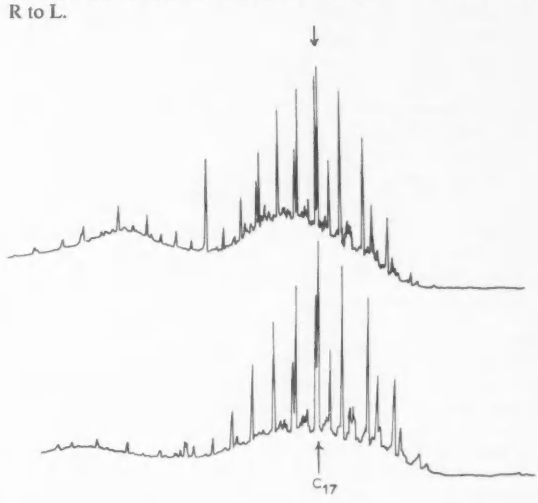


FIG. 3. (*top*) Gas chromatogram of sediment sample from station 6A (1971); OV-1 SCOT column. (*bottom*) Gas chromatogram of sediment sample from station 32 (1976); SE-30 SCOT column.

served 5 yr earlier. The stations located on the west shore of the cove were of prime interest because of previous pathological studies (Barry and Yevich 1975) and were, therefore, considered as a unit in detail. This site series commences with 1971 station 1A located on the extreme southwest shore and continues to site 13A located on the northwest shore beyond culvert No. 3 (Fig. 1). While there is a considerable variation in the hydrocarbon distribution among individual 1971 sites along the west shoreline, the 1976 data correlated closely with the 1971 measurements at a large majority of the stations.

The sites containing the least weathered material were

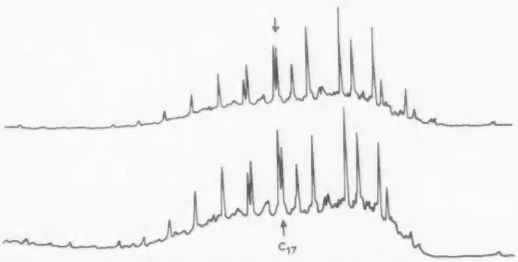


FIG. 4. (*top*) Gas chromatogram of sediment sample from station 7B (1972); SE-30 SCOT column. (*bottom*) Gas chromatogram of sediment sample from station 37 (1976); SE-30 SCOT column.

established and examined in detail. These stations were identified as 5A [23] and 6A [32] in the 1971 collection and 7B [37] in the 1972 collection (Fig. 2–4). The corresponding 1976 stations are identified by the numbers enclosed in brackets following the earlier site registration figures.

It is noteworthy that in comparing the packed column data with the SCOT column data, the two important isoprenoids pristane ($C_{19}$) and phytane ($C_{20}$) are interchanged with their comparison linear chain peaks ($C_{17}$ and $C_{18}$). In the Apiezon L chromatograms the isoprenoids, therefore, preceed the $C_{17}$ and $C_{18}$ peaks while in the SE-30 and OV-1 chromatograms the isoprenoids trail the $C_{17}$ and $C_{18}$ peaks. All chromatograms are normalized to the $C_{17}$-pristane doublet.

The weathered condition of the petroleum residues isolated from the marine sediments examined in this study is qualitatively judged by several features found in the gas-chromatographic data. For example, in a relatively unweathered refined No. 2 or JP-4 oil one would expect to find (1) that the background envelope profile will be low compared to the linear chain hydrocarbon peaks and that there will be little or no secondary high molecular weight (high temperature) envelope; (2) linear chain hydrocarbon peaks will be considerably more intense relative to the isoprenoid bands (based on comparison to standard unweathered samples); and (3) the primary low molecular weight (low temperature) front end of the chromatogram will not have been eliminated by volatilization and dissolution. All three of the sites presently under consideration possessed material with moderate to low background envelopes (Fig. 2–4), high linear chain intensities, and all but the 1976 sample from station 37, exhibited a $C_{12}$ linear peak (station 37 starts the homologous series at $C_{13}$). Of the two stations contained in the 1971 set, 6A was judged to be slightly less weathered than 5A. This assessment of sample condition was based simply on a qualitative estimate of the relative intensities of the linear chain and isoprenoid peaks (Fig. 2 and 3). It is particularly noteworthy that this same relationship still exists at the corresponding stations 32 and 23 in 1976.

The hydrocarbon residue isolated from site 7B in July 1972, over a year following the collections at stations 5A and 6A, was considered to be somewhat more weathered than material obtained from either 5A or 6A (this weathered condition was initially ascribed (Mayo et al. 1972) to the time interval between the collections at 7B and 5A or 6A; however, see later discussion for a reassessment of this interpretation) and this relative ranking in weathered condition is still maintained with the 1976 sediment sample [37]. In addition, it appears that residues obtained from this latter station, [37], do appear to be slightly more aged than the earlier sample (7B) collected near this location, as the later material has lost all of the $C_{12}$ and most of the $C_{13}$ linear chain peak intensities (Fig. 4). These latter results are consistent with the fact that this site was placed in an area with slightly better drainage. Station 7B is located on the lower elevations of a domed section of the intertidal zone.

Gross hydrocarbon concentrations observed at the three locations in 1976 were quite consistent with the 1971 values. The recorded quantities ranged from slightly below 100 ppm to slightly above 200 ppm (see Table 1), with two stations down and one up in concentration.

It should be emphasized that sites 5A, 6A, and 7B are located very close to each other and lie either directly in the drainage plain of culvert no. 2 or on the edge of this system (see Fig. 1). This culvert was previously identified as the prime source of the 1971 spill (Mayo et al. 1972) (upland sediments still containing high concentrations of oil drain through this culvert).

The next stations to be examined in the analysis are 7A and 4A which are located respectively equidistant just to the north and the south of the no. 2 culvert drainage system (sites 5A, 6A, and 7B). The residues isolated from these sites were found to have undergone more extensive weathering compared with those petroleum fractions collected at sites 5A, 6A, or 7B. These more removed sites, however, exhibit very nearly identical hydrocarbon distributions relative to each other (see Fig. 5 and 6). Station 4A [16] does appear to be slightly less weathered than 7A [47] as judged by the presence of a slightly higher low molecular weight (front end) envelope and slightly more intense linear chain peaks relative to the envelope.

At all stations examined in this study the relative quantities of pristane, phytane, and linear chain $C_{17}$ and $C_{18}$ hydrocarbons were examined with considerable care as this aliphatic series should be the least affected by weathering processes (Zafiriou et al. 1972). In this regard, it was particularly striking to observe that the 1976 relative concentrations of these compounds are virtually identical to those measured in 1971, and for example, a slight excess of pristane over $C_{17}$ at site 4A compared to site 7A is still noted at site [16] compared to site [47] in 1976.

Moving farther away from the spill source to the north and south along the west shore, stations 13A [98]

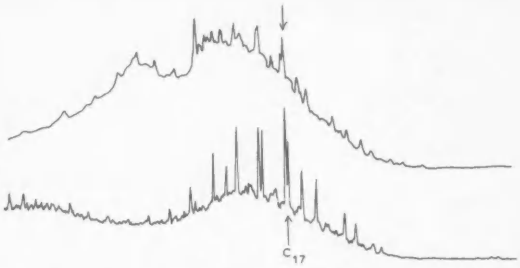


FIG. 5. (*top*) Gas chromatogram of sediment sample from station 4A (1971); Apiezon-L packed column. (*bottom*) Gas chromatogram of sediment sample from station 16 (1976); SE-30 SCOT column.

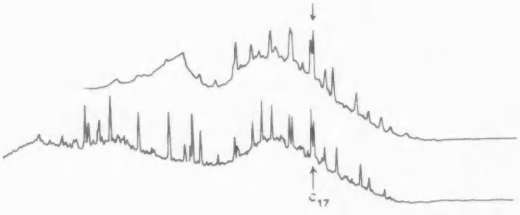


FIG. 6. (*top*) Gas chromatogram of sediment sample from station 7A (1971); Apiezon-L packed column. (*bottom*) Gas chromatogram of sediment sample from station 47 (1976); SE-30 SCOT column.

and 3A [11] (Fig. 7 and 8) were next compared. Both of these stations appear to contain residues that have weathered to an extent not greatly different from the two stations just discussed (4A [16] and 7A [47]); compare Fig. 5 and 6 with Fig. 7 and 8. As at the other sites examined in this study, the 1976 samples collected at these stations possess nearly identical pristane–phytane and $C_{17}$–$C_{18}$ relative concentrations when compared to 1971 values for these sites. In addition, the gas-chromatographic envelope profiles of these residues are very similar (see Fig. 7 and 8).

The gross hydrocarbon concentration values (see Table 1) continued to correlate closely between 1971 and 1976, as both 3A and [11] possess less than 50 ppm and 13A and [98] both contain approximately 250 ppm.

Three other comparison sites were established for detailed examination on the west shore of the Cove:

1) Site 10A [83] located nearly midway between 7A [47] and 13A [98] compares very well (see Fig. 9) with respect to the four basic criteria employed in determining identities at the other sites, i.e. (a) pristane, phytane — $C_{17}$, $C_{18}$ ratios; (b) isoprenoid — linear chain ratios; (c) envelope — linear chain ratios; (d) and envelope profile.

Relative to the other west shore stations, it appears, as might have been expected, from the developing picture of this area, that this station is intermediate in contamination between sites 7A [47] and 13A [98]. The

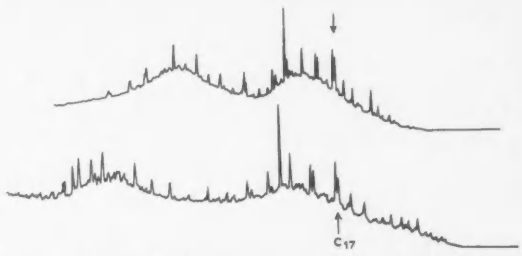


FIG. 7. (*top*) Gas chromatogram of sediment sample from station 3A (1971); OV-1 SCOT column. (*bottom*) Gas chromatogram of sediment sample from station 11 (1976); SE-30 SCOT column.

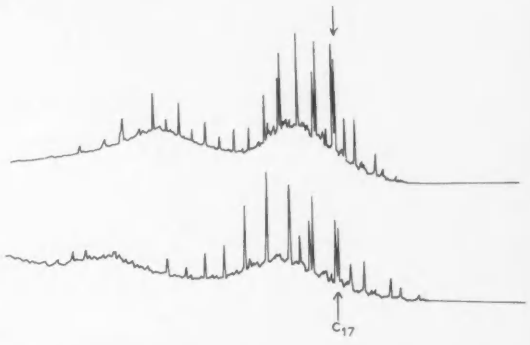


FIG. 8. (*top*) Gas chromatogram of sediment sample from station 13A (1971); OV-1 SCOT column. (*bottom*) Gas chromatogram of sediment sample from station 98 (1976); SE-30 SCOT column.

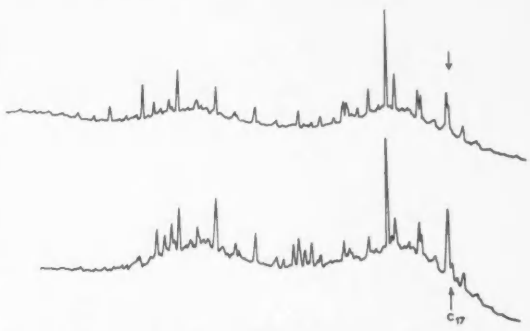


FIG. 9. (*top*) Gas chromatogram of sediment sample from station 10A (1971); SE-30 SCOT column. (*bottom*) Gas chromatogram of sediment sample from station 83 (1976); SE-30 SCOT column.

material isolated from this section of the shore does appear to have lost slightly more of the low temperature portion of the envelope than that from the other northwest shore sites. It is particularly noteworthy that while the relative depletion of the front end of the chromatograms at site 10A introduces a slight discontinuity in

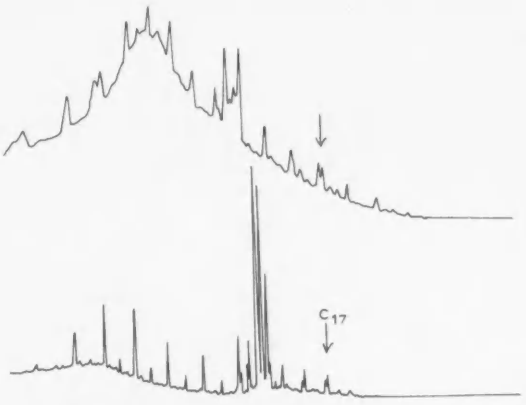


FIG. 10. (*top*) Gas chromatogram of sediment sample from station 8B (1972); Apiezon-L packed column. (*bottom*) Gas chromatogram of sediment sample from station 55 (1976); SE-30 SCOT column.

the weathering sequence of stations north of the culvert, this site perturbation still continues to be observed in the samples collected in this area in 1976.

2) Site 8B [55] is located on a well-drained "dome-like" structure (see also site 7B) composed of mixed gravel and clay. The station also lies in the upper portion of the intertidal zone and is in close proximity to culvert no. 2, but because of the domed nature of its location it is not influenced by drainage from this latter system. Furthermore, this station was unique to the west shore as it showed only trace contamination by light refined oil in 1971. A modest amount of heavily weathered residual oil appeared to be present in the area at that time — 82 ppm (see high temperature envelope in Fig. 10). The site still indicates no contamination by light refined oil and at present possesses only a small amount of material (6 ppm) identified as predominantly natural hydrocarbons. It is noteworthy that a dominant contribution to the 1971 petroleum portion of the residue could easily have resulted from the incorporation of a single small tar ball during collection of the sediment sample at high beach elevations. Support for this latter suggestion is the fact that site 8B is the only station studied on the west shore in which apparent leaching from marine sediments appears to have significantly reduced the residue content during the 5-yr period (see Table 1). It should be emphasized again that light refined hydrocarbons do not appear to have been a contaminating factor at this station for any extended period of time during these studies.

3) Site 1A [2] is the only other location on the west side of the Cove that exhibits any measurable departure from a gas-chromatographic match between 1971 and 1976 sediment residues (see Fig. 11). While gross concentrations in this area have remained approximately the same (see Table 1) during the 5-yr time interval, examination of the envelope structure indicates that a significant shift from light to heavy hydrocarbon distribution has occurred. As the total residue quantities have not changed significantly, it would appear that this profile shift is not simply the result of low molecular weight material being leached out preferentially. Furthermore, the linear chain hydrocarbon peaks are somewhat more intense relative to the background envelope in the 1976 residues. This latter observation is a further argument against simple leaching effects. The most likely postspill hydrocarbon influence on this region is a commercial docking facility located at the extreme southwestern end of the cove (see Fig. 1) and station 1A [2] is the site closest to that operation. Small amounts of bunkering oil reaching this site in the form

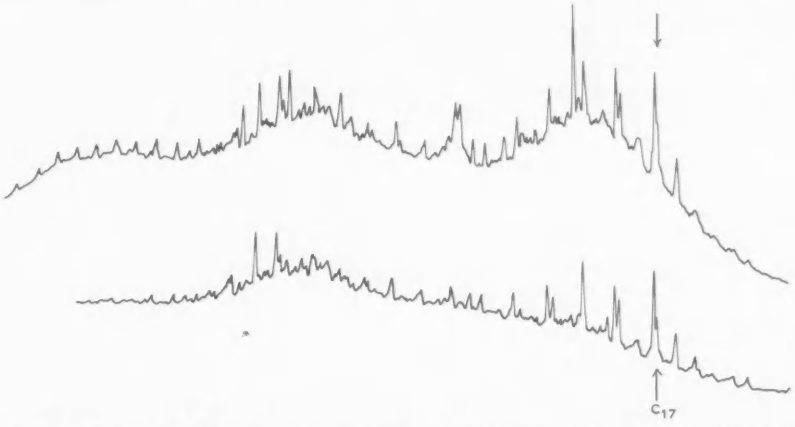


FIG. 11. (*top*) Gas chromatogram of sediment sample from station 1A (1971); SE-30 SCOT column. (*bottom*) Gas chromatogram of sediment sample from station 2 (1976); SE-30 SCOT column.

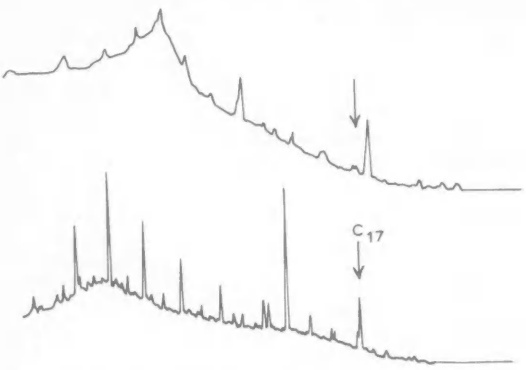


FIG. 12. (*top*) Gas chromatogram of sediment sample from station 16A (1971); Apiezon-L packed column. (*bottom*) Gas chromatogram of sediment sample from station 149 (1976); SE-30 SCOT column.

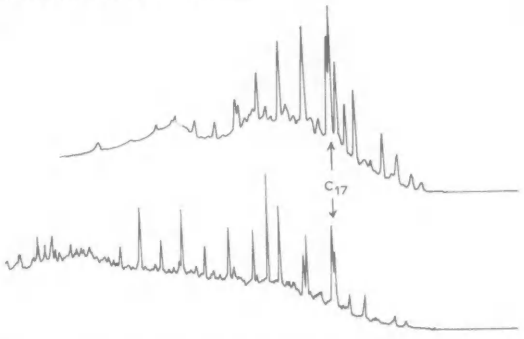


FIG. 13. (*top*) Gas chromatogram of sediment sample from station 17A (1971); Apiezon-L packed column. (*bottom*) Gas chromatogram of sediment sample from station 158 (1976); SE-30 SCOT column.

of tar balls could easily have produced the observed changes in envelope content. Another possible, although much less likely, source of subsequent hydrocarbon contamination of this station is that it lies directly in the drainage plain of culvert no. 1 which has been shown (see later discussion) to contain minor quantities of weathered petroleum residues.

The east shore of the Cove was demonstrated in earlier investigations to be much less involved in the light refined spill (Dow 1972). At that time only two locations indicated the presence of fresh oil and most of the northeast shoreline possessed bare traces of material.

The very close intrasite matching between 1971 and 1976 petroleum residues observed at the west shore stations, however, continues on the east shore. For example, station 16A [149] shows the 1971 and 1976 samples (Fig. 12) to be nearly identical, even to the point of including the same distribution of the natural biogenic compounds.

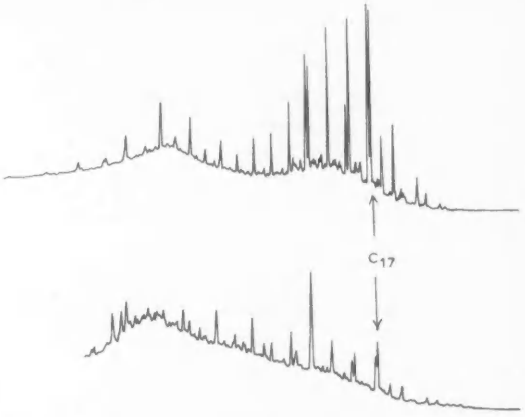


FIG. 14. (*top*) Gas chromatogram of sediment sample from station 18A (1971); OV-1 SCOT column. (*bottom*) Gas chromatogram of sediment sample from station 168 (1976); SE-30 SCOT column.

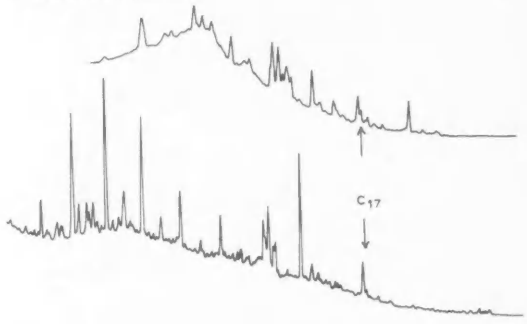


FIG. 15. (*top*) Gas chromatogram of sediment sample from station 22C (1972); Apiezon-L packed column. (*bottom*) Gas chromatogram of sediment sample from station 26D (1976); SE-30 SCOT column.

Both of the locations (sites 17A [158] and 18A [168]) on the northeast shore that had light petroleum hydrocarbons in 1971 were reexamined in the 1976 study. These sites still evidence petroleum residues of light refined origin and they still compare very favorably to the 1971 gas-chromatographic distribution (see Fig. 13 and 14). Some preferential degradation of the linear chain hydrocarbons relative to the isoprenoids, a situation not observed to be taking place on the west shore, was detected at the contaminated stations on the east side of the Cove. Gross concentrations at 18A [168] were, on the other hand, still quite comparable to 1971 values (see Table 1). There does appear, however, to have been a considerable amount of material leached from the 17A [158] station during the 5-yr period.

Several dredge samples were collected from the center of the Cove during 1976. These samples were chosen to correspond to the same sample areas where

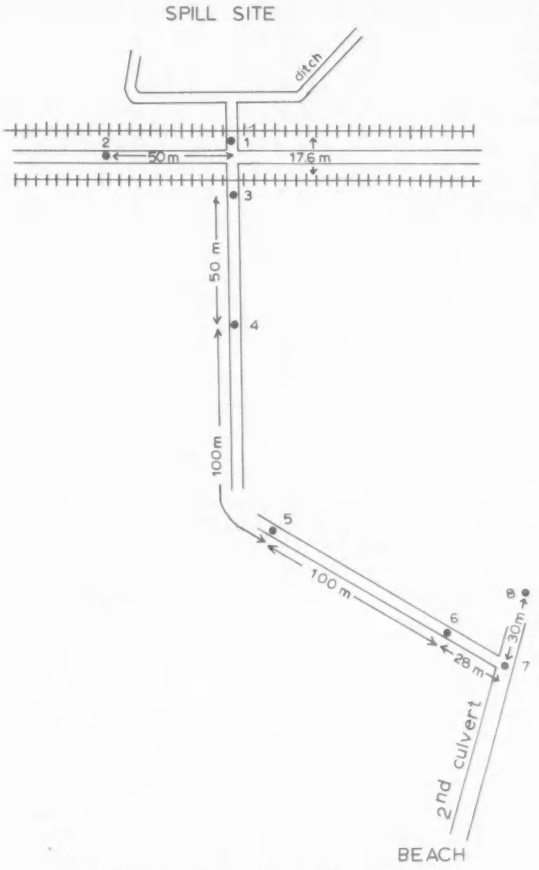


FIG. 16. Upland sampling stations in 1976 study.

subtidal samples were collected in 1972 (Mayo et al. 1972). As all of the subtidal material collected from the Cove appeared to be very similar in both collections and as great similarities were observed within each set including gross concentrations only a single example (22C [26D]) was considered in detail (Fig. 15). The gas-chromatographic data indicate that a considerable number of hydrocarbons of biogenic origin are present in these subtidal residues along with a small quantity of material giving rise to an unresolved envelope that very likely represents heavily weathered residues to which the 1971 spill could not have been a major contributor. Among the series of natural compounds of significant concentration there occurs a collection between $C_{20}$–$C_{21}$ which are reproduced in both sets of samples.

During the 1976 reexamination of Long Cove a thorough investigation of the upland sediments immediately adjacent to the tank farm and leading to the shore line was undertaken to determine the extent of the present contamination of the area and to establish

TABLE 2. Gravimetric results for the extraction of hydrocarbon residues from sediment samples taken from the upland stations leading to culvert 2 (sites 1–8), culvert 3 (sites 9 and 10), and culvert 1 (sites 11 and 12). Values are given in parts per million (ppm) dry weight basis. All samples taken on August 19, 1976.

| Station number | ppm aliphatic | ppm aromatic | ppm total |
|---|---|---|---|
| 1 | 1647 | 602 | 2249 |
| 2 | 1786 | 299 | 2085 |
| 3 | 1811 | 338 | 2149 |
| 4 | 1095 | 290 | 1385 |
| 5 | 712 | 667 | 1379 |
| 6 | 6291 | 834 | 7125 |
| 7 | 7 | 13 | 20 |
| 8 | 6 | 12 | 18 |
| 9 | 100 | 139 | 239 |
| 10 | 17 | 82 | 99 |
| 11 | 155 | 77 | 232 |
| 12 | 3 | 6 | 9 |

whether upland leaching was continuing to have an impact on the lower elevation marine sediments. Of the three drainage systems leading from the fuel depot and emptying onto the west shore only the one utilizing culvert no. 2 appeared to be involved in the 1971 spill, and indeed, only that area was found to possess significant quantities of light refined petroleum hydrocarbons in 1976. Eight sampling stations were eventually established along storm ditches (see Fig. 16) leading to the no. 2 culvert from the tank farm. Six of these sites were subsequently shown to possess hydrocarbon concentrations in excess of 1000 ppm (see Table 2). Of particular historical interest, were the results of the analysis of upland stations 1 and 2 which are located closest to the point of the spill source and which were positioned in two separate ditches leading from the tank farm to the main drainage ditch. Station 1 unquestionably still contains large quantities (1647 ppm) of jet aviation fuel, JP-4. As JP-4 was the fuel being handled at the fuel depot in 1971, we have assumed that the material isolated at upland station 1 represents a partially weathered sample of JP-4 derived from the original spill (see Fig. 17). Note that although the linear chain hydrocarbon concentrations still exceed the isoprenoid hydrocarbon series that the material contained in this site has weathered down to $C_{12}$, whereas in 1971 hydrocarbons with molecular weights as low as $C_8$ were easily observed in the water column draining the area (see Fig. 17). There is, however, no evidence at all for the presence of even traces of No. 2 oil at this station. On the other hand, at upland station 2, large quantities (1786 ppm of similar magnitude when compared to the concentrations found at upland station 1), of material that clearly is a weathered ($C_{14}$ identified, see Fig. 18) No. 2 oil type petroleum residue were observed. Furthermore, at this site no traces of JP-4 could be detected. These observations suggest that

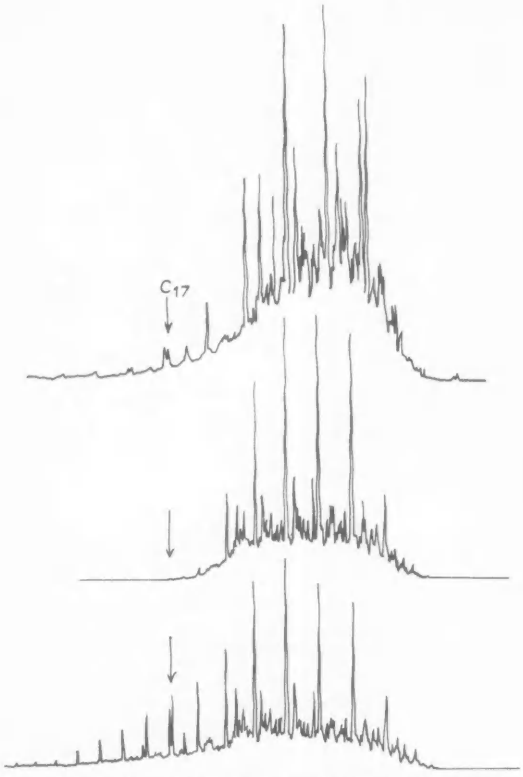


FIG. 17. (*top*) Gas chromatogram of sediment sample from upland station 1 (1976); SE-30 SCOT column. (*middle*) Gas chromatogram of jet fuel reference sample (1972); OV-1 SCOT column. (*bottom*) Gas chromatogram of extract from water sample taken from culvert 2 (1971); OV-1 SCOT column.

the assessment of the source of the 1971 spill may have been more complicated than was initially reported (rupture of a line simultaneously carrying both JP-4 and No. 2 oil) (Dow 1972). The two ditches (containing upland stations 1 and 2) join each other just before upland stations 3, 4, 5, and 6 which are located in the combined drainage system (see Fig. 16). These latter stations contain very large quantities of petroleum residues; one site, in fact, possesses in excess of 7000 ppm (upland site 6) hydrocarbons. These lower terrestial elevation sites were shown by gas-chromatographic analysis to possess material derived from upland stations 1 and 2. Based on envelope profile heights it appears that at upland stations 4 and 5, the No. 2 oil residues exceed the kerosene residues (see Fig. 19). At upland station 6 the kerosene residues appear to exceed the No. 2 oil residues (see Fig. 19). At all sites, however, with the single exception of upland station 1 the relative concentrations of the isoprenoid hydrocarbons exceed the linear chain hydrocarbon concentrations by

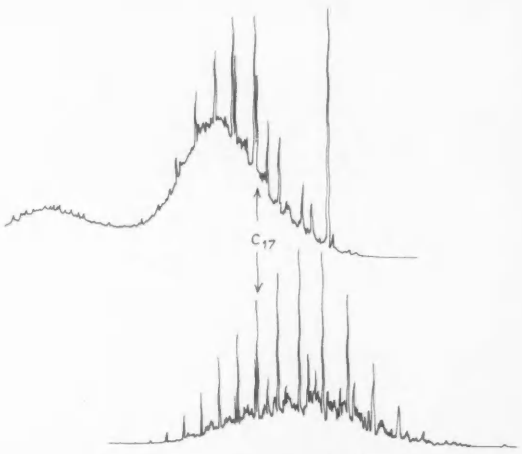


FIG. 18. (*top*) Gas chromatogram of sediment sample from upland station 2 (1976); SE-30 SCOT column. (*bottom*) Gas chromatogram of No. 2 fuel oil reference sample (1971); OV-1 SCOT column.

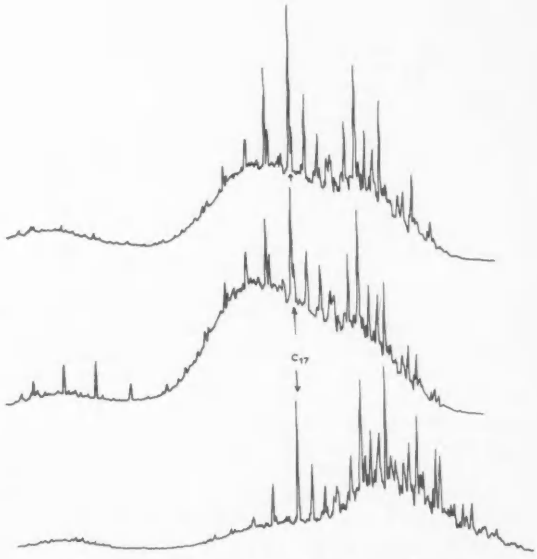


FIG. 19. (*top*) Gas chromatogram of sediment sample from upland station 4 (1976). (*middle*) Gas chromatogram of sediment sample from upland station 5 (1976). (*bottom*) Gas chromatogram of sediment sample from upland station 6 (1976); All SE-30 SCOT column.

a margin not observed in any of the marine sediments examined on the west shore of Long Cove.

Upland stations 7 and 8 did not possess significant quantities of petroleum hydrocarbons. Upland site 7, although located in the drainage system leading to the second culvert, apparently had been leached clean by

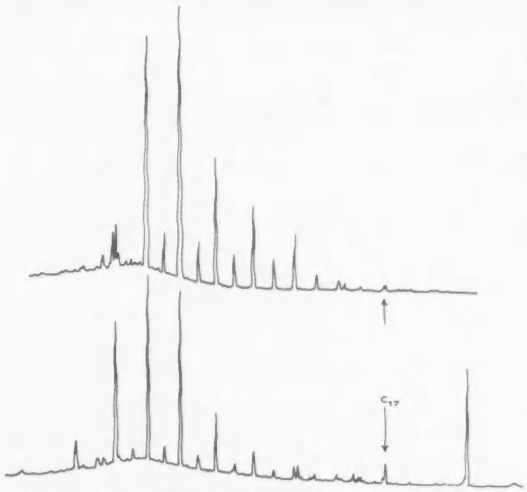


FIG. 20. (*top*) Gas chromatogram of sediment sample from upland station 8 (1976). (*bottom*) Gas chromatogram of sediment sample from upland station 9 (1976); both SE-30 SCOT column.

another intersecting drainage ditch not containing any petroleum residues, as only traces of heavily weathered No. 2 oil were identified in the surface sediments at this site. At upland station 8 located on the spur ditch which intersected the main ditch at upland site 7, no petroleum hydrocarbons were found. A small amount of leaf wax was identified in the residue isolated from this location (see Fig. 20).

In the third culvert drainage system only natural hydrocarbons, mainly leaf waxes peaking at $C_{29}$, could be identified (see Fig. 20). In the first culvert drainage system the results were nearly identical to the third culvert area observations. Of the two upland stations located in this latter drainage system, one (11) showed only natural hydrocarbons while the other one (12) possessed small traces of a weathered No. 2 oil mixed with natural hydrocarbons of biogenic origin.

The repopulation of the Cove by *Mya* is proceeding very slowly (a detailed discussion of this observation is given in Gilfillan et al. 1977). Indeed, these animals were found only in the higher elevations of the intertidal zone which are particularly well drained. An example of this type of location is site 8B [55] (see Fig. 1).

The detailed sediment studies of 1976 have led to gross hydrocarbon concentration data covering the entire intertidal area of the Cove which in turn has allowed the construction of 50- and 100-ppm concentration contour lines for the Cove. In only one limited area was the presence of clams identified in a section exceeding 100 ppm. This area was located on the fringe of the drainage plain of culvert 2. Close examinations of these marine sediments indicated that the upper 5–8 cm of the material was often leached clean of petroleum residues and that clams were found at several stations where this cleansing action had occurred. The more mature adult clams found at the stations possessing living juveniles, however, were very often observed to be dead. These latter animals had reached a size range where the vertical dimension of their burrow had penetrated into the subsurface sediments which still remained contaminated with petroleum residues. In addition to the problems encountered at 100 ppm, very few clams were observed living in areas possessing residue concentrations of 50–100 ppm range in which the major contribution to the material was of petroleum origin.

## Conclusions

By utilizing the data obtained from the 1976 reinvestigation of Long Cove in combination with earlier studies, it has been possible to develop a somewhat clearer understanding of the transport and weathering processes perturbing the spill area in Searsport.

First, it appears, somewhat surprisingly, but not totally unexpectedly, that in the Cove, which possesses mainly cold anoxic fine sediments, very slow leaching and weathering of the light refined residues is taking place. This assessment is based on the observation that both the gross hydrocarbon concentrations and relative hydrocarbon compound distribution (assessed by gas chromatography) have changed very little at the large majority of comparison sites during the 5-yr period, 1971–76.

Second, as all but one of the upland sites (the one exception is at the highest elevation and closest to the spill site) studied exhibit a larger depletion of the linear chain hydrocarbons relative to the isoprenoid series than do the marine sediments on the west shore, it can be concluded that terrestial leaching at the present time has only a relatively minor influence on the concentration of petroleum residues found in these latter sediments. Indeed, the data unquestionably suggest that while microbiological degradation of the aliphatic linear chain systems appears to have had a measurable impact on the residues contained in upland sediments this action is greatly suppressed in the residues absorbed on the anoxic cold clay silt of the Cove.

Third, it is now possible to suggest as a result of this study that the dominant factor controlling the distribution of hydrocarbons in the marine sediments of Long Cove over the past 5 yr was the physical weathering of the oil by evaporation and dissolution during transport from the immediate upland spill location to the marine sediment sites in question during the early phases of the spill. In fact, following sediment penetration at the individual site locations, weathering of these particular petroleum residues must have decreased very rapidly to nearly zero. This latter observation is based on the large changes in relative compound distribution observed between the upland stations and marine sediment stations and the lack of any significant change in

gas-chromatographic profiles of the marine stations over the intervening 5-yr period between the first and second studies.

Fourth, repopulation of the Cove by *Mya arenaria* has correlated closely with intertidal sediment hydrocarbon concentrations. At four exceptional sites, clams were located in an area with a concentration in excess of 100 ppm. At only six additional stations were *Mya* present at sites possessing between 50- and 100-ppm hydrocarbon residues. In the remaining 44 stations currently occupied by soft-shelled clams in Long Cove, petroleum hydrocarbons were found to be present in concentrations of 49 ppm or less.

Based on the above data the intertidal sediments of Long Cove will apparently continue to possess contaminating concentrations of petroleum hydrocarbon residues for a substantial period. As a result of the very slow decline in these hydrocarbon concentrations, the restoration of clam populations to prespill levels will also continue to be suppressed.

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# Mechanical Dispersal of Oil Stranded in the Littoral Zone[1]

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OWENS, E. H. 1978. Mechanical dispersal of oil stranded in the littoral zone. J. Fish. Res. Board Can. 35: 563–572.

The natural weathering and dispersion of oil stranded in the littoral zone depend on the type and volume of the oil and on the amount of available energy. Energy inputs can be biological, chemical, mechanical, or thermal; however, the single most important input appears to be from mechanical energy (winds, waves, tides, water levels, and ice). The residence time or persistence of stranded oils increases as mechanical energy levels at the shoreline decrease. The primary inputs of mechanical energy at the shoreline are due to wave action, and energy levels vary depending on the wind regime, fetch areas, and local exposure of a section of coast. In cold or polar climates, energy levels at the shoreline are reduced by the presence of ice on the sea or an ice foot on the beach. This review of the concepts associated with littoral processes, in terms of the mechanical dispersal of stranded oil, provides the basis for estimation of the persistence of oil on shorelines.

*Key words:* petroleum (weathering, dispersal), coastal processes, wave energy, sea ice, ice foot, Canada

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L'altération naturelle à l'air et la dispersion du pétrole échoué dans la zone littorale dépend du type et du volume de pétrole ainsi que de la quantité d'énergie disponible. Les apports énergétiques peuvent être biologiques, chimiques, mécaniques ou thermiques; cependant, le facteur le plus important semble être l'énergie mécanique (vents, vagues, marées, niveaux d'eau et glace). Le temps de résidence ou de persistance du pétrole échoué diminue en même temps que diminuent les niveaux d'énergie mécanique à la ligne de rivage. Les principaux apports d'énergie mécanique à la ligne de rivage proviennent de l'action des vagues et les niveaux d'énergie varient selon le régime des vents, les zones de génération et l'exposition locale d'un secteur de la côte. Dans les climats froids et polaires, la présence de glace sur la mer ou d'un banc de glace sur le rivage abaisse les niveaux d'énergie à la ligne de rivage. Cette revue des concepts associés aux processus du littoral, en fonction de la dispersion mécanique du pétrole échoué, fournit une base d'estimation de la persistance du pétrole sur les rivages.

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THE physical properties and chemical composition of spilled oil change through time as a result of weathering and aging. Degradation processes (primarily biodegradation, dissolution, evaporation, and photooxidation) result in the eventual breakdown and dispersal of the spilled oil. The rate at which oil stranded on a shoreline is dispersed and removed depends on the initial composition and volume of the oil, on the ratio of the exposed surface area to the total volume of the oil, and on the available energy in the littoral zone. To date research has been focused largely on the biological, chemical, and physical processes associated with weathering and aging and generally has been related to a specific spill or to laboratory analyses. However, relatively little attention has been given to the role and effects of local and regional variations in coastal processes and mechanical energy levels.

The amounts of available energy can vary, depending on the inputs from mechanical (winds, waves), thermal (air and water temperatures), biological (microbial degradation), or chemical (photooxidation) sources. This review considers the role of natural shoreline cleaning from the point of view of the effects of mechanical energy on the persistence of stranded oil.

The importance of mechanical energy inputs has been demonstrated by Betancourt and McLean (1973) from an analysis of stranded Bunker C oil spilled from the tanker *Arrow* in Chedabucto Bay, Nova Scotia. Their results show that the weathering of oil stranded on the shoreline, above the limit of wave activity, was not great in terms of the total loss of material, 20% by weight (Fig. 1) and that "after one year changes in composition were almost negligible indicating that the

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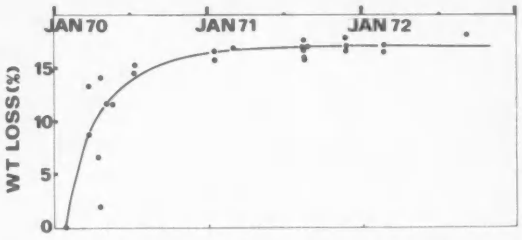



FIG. 1. Variation in weight loss of weathered Bunker C oil with time. Samples taken from Chedabucto Bay after the *Arrow* spill (from McLean and Betancourt 1973).

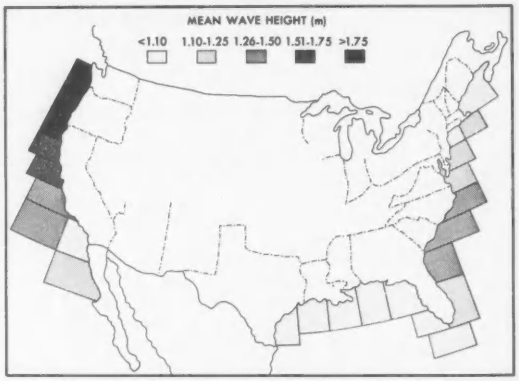



FIG. 2. Mean annual wave-height values for the coasts of the United States (from Thompson and Harris 1972).

weathering process had almost stopped." These authors suggest that further dispersal would be related to inputs of mechanical energy, such as abrasion or erosion.

Analyses by Rashid (1974) of the physical and chemical properties of oil from the *Arrow* spill stranded in the intertidal zone also support the hypothesis that the level of mechanical energy at the shoreline has a major effect on rates of degradation. Rashid notes that in a low-energy environment "the residual oils . . . may remain relatively unaltered for years" but that in high wave-energy environments "natural processes (i.e. waves) would have restored the beaches by the physical and chemical degradation of the oils." Analytical results (Table 1) from samples collected 3½ yr after the spill, at a site protected from wave action at all times, show a low viscosity and a high hydrocarbon content. Comparison with original and stored samples of the Bunker C oil indicates that the extent of degradation was only slightly more than that of the samples stored in the laboratory. By contrast, on exposed beaches subject to continual wave action, the oil residues were highly viscous and had a high resin–asphaltene content, suggesting considerable degradation. In high wave-energy environments the "weathering forces change the composition of the oil substantially and natural processes clean the beach rapidly" (Owens and Rashid 1976).

To define the effects of coastal processes on the degradation and dispersal of oil stranded in the littoral zone, I will discuss several major factors. Probably the most critical of these is the role of mechanical energy in the form of winds, waves, tides (or water levels), and ice. The inputs of mechanical energy associated with these processes control the nature and the rate at which changes in the littoral zone take place. Stranded oil is dispersed by these processes, and this dispersal has a critical impact on the chemical composition and the physical properties of the oil by exposing larger surface areas to weathering and aging.

Table 2a indicates the relationships between degradation processes, energy sources, and the properties of spilled oil. The levels of mechanical energy at the shoreline depend primarily on coastal processes but it is also necessary to consider shoreline conditions such as sediment size and rock type (Table 2b) to adequately describe the nature and the rates at which changes take place. Also, the presence of oil in the littoral zone has an effect on the dynamic relationship between the coastal processes and the shoreline factors.

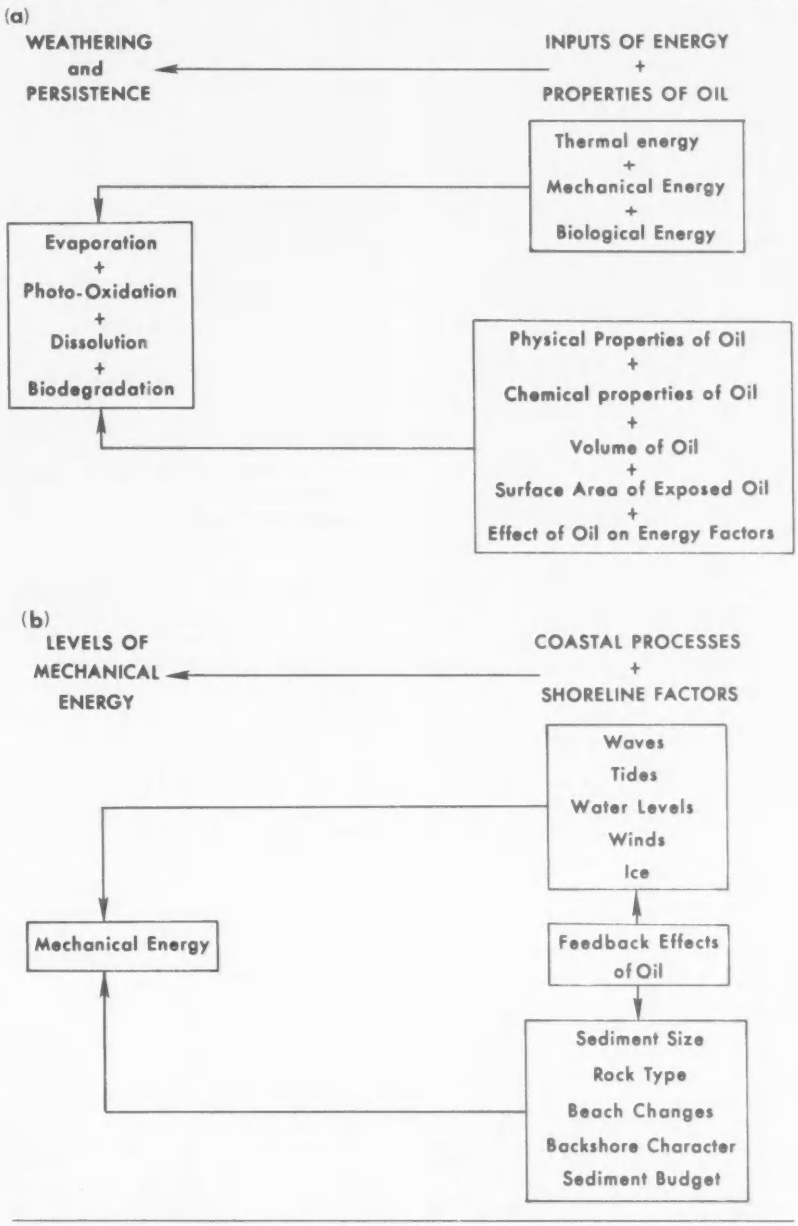
## Coastal Processes and the Persistence of Stranded Oil

Coastal processes include winds, waves, tides, and the effects of ice, but the action of wind-generated surface waves is the most common form of energy at the shoreline. Inman (1973) states that a 1-m-high wave transmits ~10 kW/m wave crest, whereas a 3-m-high wave has the power of 100 kW/m wave crest. On a regional scale, levels of wave energy on open coasts vary considerably. Figure 2 indicates the mean annual observed wave height for coastal regions of the United States. On a general basis it can be assumed that owing to the difference in wave-energy levels, oil stranded in

TABLE 1. Chemical and physical characteristics of original and residual Bunker C oils extracted from surface sediments collected 3½ yr after the spill in Chedabucto Bay. (From Rashid 1974.)

| | Original | Stored sample | Low-energy coast | Moderate-energy coast | High-energy coast |
|---|---|---|---|---|---|
| Total hydrocarbons (%) | 73.1 | 51 | 49 | 47 | 34 |
| Asphaltenes (%) | 16.3 | 20 | 22 | 23 | 22 |
| Resins and NSO's (%) | 10.6 | 29 | 29 | 30 | 44 |
| Viscosity (cP) | — | 19 584 | 28 600 | 1 210 000 | 3 640 000 |

TABLE 2. Factors that affect oil degradation and inputs of mechanical energy (from Owens 1977b).


(a)
WEATHERING and PERSISTENCE
INPUTS OF ENERGY + PROPERTIES OF OIL
Thermal energy + Mechanical Energy + Biological Energy
Evaporation + Photo-Oxidation + Dissolution + Biodegradation
Physical Properties of Oil + Chemical properties of Oil + Volume of Oil + Surface Area of Exposed Oil + Effect of Oil on Energy Factors
(b)
LEVELS OF MECHANICAL ENERGY
COASTAL PROCESSES + SHORELINE FACTORS
Waves
Tides
Water Levels
Winds
Ice
Mechanical Energy
Feedback Effects of Oil
Sediment Size
Rock Type
Beach Changes
Backshore Character
Sediment Budget

the littoral zone on the coasts of Oregon and Washington would be dispersed more rapidly than oil on the coasts of the Gulf of Mexico.

In terms of local persistence times of stranded oil, this simplified overview must be modified to allow for small-scale variations in energy levels at the shoreline, owing to differences in exposure or to wave sheltering that result from coastal configuration and beach orienta-

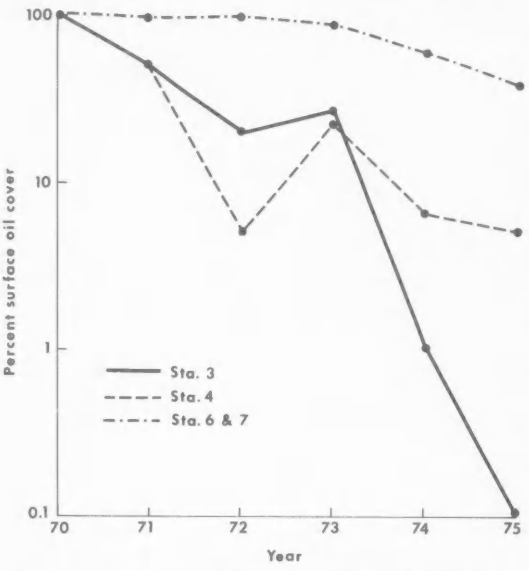



FIG. 3. Surface oil cover at the high watermark from stations in northern Chedabucto Bay, 1970–75 (from Thomas 1977).

tion. In Chedabucto Bay, which has a very irregular shoreline, Thomas (1977) measured the surface oil cover near the high watermark from 1970 to 1975 at four stations within 15 km of each other and reported a large range in values (Fig. 3). In terms of levels of wave activity, station 3 is a moderately exposed location and station 4 is a sheltered site, both in Arichat Harbour, and stations 6 and 7 are very sheltered shorelines in Janvrin Lagoon. Thomas notes that the results demonstrate "the faster disappearance of oil in proportion to exposure."

A smaller scale survey of surface oil cover by Owens and Rashid (1976), in the Black Duck Cove area adjacent to Chedabucto Bay, showed that after 3 yr the Bunker C oil that had been stranded on an exposed boulder spit was abraded from the intertidal zone (Fig. 4). Observations showed that the contaminated intertidal zone was in fact cleaned within 4 mo of oil reaching the beach. Above the limit of normal wave activity (the supratidal zone) a band of oil remained but was being buried as cobbles and boulders were thrown up onto the highest parts of the beach (Fig. 5). In the adjacent lagoon a 100% oil cover (10–25 cm thick) remained in many sections of this very sheltered environment (Fig. 6).

Oil that is stranded on the coast in the intertidal zone of a high wave-energy environment is rapidly dispersed by the hydraulic action of breaking waves or swash and backwash. This self-cleaning process can occur within only a few weeks. By contrast, oil that is stranded in low wave-energy environments, such as lagoons or sheltered bays, can persist for periods in the order of tens of years (Vandermeulen and Gordon 1976). In this latter case the dispersal of the oil depends primarily on the effects of thermal, biological, or chemical processes, as little mechanical energy is available for the removal of oil from the shoreline.

The most important effect of the hydraulic action of waves on a coast is the abrasion, dispersal, and redistribution of sediments and oil. The amounts of sediment or oil that are redistributed by wave action are

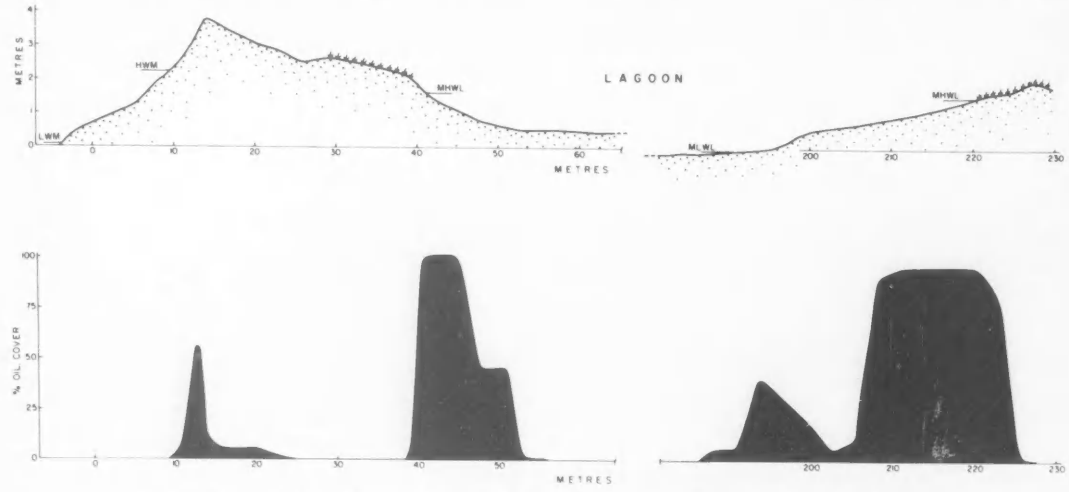



FIG. 4. Topographic profile (*upper*) and surface oil cover (*lower*) across the spit and lagoon at Black Duck Cove, N.S., June 1973. The oil cover was measured on a 1-m-wide transect along the line of the topographic profile 3 yr after Bunker C oil was stranded at this location (from Owens and Rashid 1976).

FIG. 5. Oil stranded above the high watermark on the outer beach of Black Duck Spit 3 yr after the *Arrow* spill, May 1973 (photo by J. R. Belanger, Bedford Institute) (c.f. Fig. 4).

related to the size of the material and to the available wave energy. As sediment size increases, so the force required to initiate and maintain transportation must increase. Oil stranded on a beach will be removed by wave action provided that (a) the oil particles are sufficiently small in size to be moved by the available wave energy, and (b) the oil does not form a complete, solid cover on the shoreline surface. If the oil is a thick continuous deposit (Fig. 7) the processes of redistribution and dispersal are slow or cease, and shoreline cleaning then depends on abrasion of the oil surface and on the mechanical breakdown of the oil cover. Once particles are abraded or broken off from the oil cover, then dispersal, transportation, and further abrasion of these particles can occur. The mechanical breakdown of stranded oil is not achieved by the force of the waves alone. The sediment that is redistributed by wave action further aids abrasion of the stranded oil.

In addition to this simple relationship between the persistence of stranded oil and the levels of wave activity, it is necessary to consider shoreline changes that result from wave-induced processes. As wave energy levels vary temporally, a beach is subject to periodic or aperiodic cycles of erosion and accretion. The timing of oil deposition on a shoreline is, therefore, important in determining the persistence and rates of natural cleaning. In general, storm waves cause erosion, and subsequent beach recovery occurs as sediment is returned by constructive wave activity. On coasts that have a large seasonal difference in wave-energy levels, a "summer–winter beach cycle" is common (Fig. 8), with erosion predominating in winter (high-energy period),

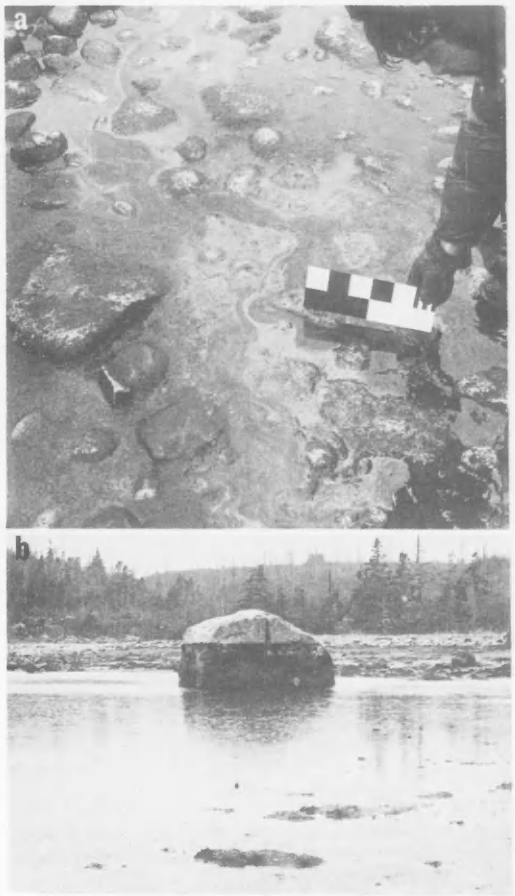



FIG. 6. Oil from the *Arrow* in the very sheltered lagoon at Black Duck Cove, May 1973, 3 yr after oil was stranded in this lagoon; a, thick (>5 cm), mobile oil in the intertidal zone; b, oil on the intertidal surface of a large rock boulder (photos by J. R. Belanger, Bedford Institute).

and accretion in summer (lower energy period). In other areas these beach changes can occur over short time periods, days or weeks, in association with storm-wave erosion and poststorm recovery. These beach cycles have a critical effect on stranded oil, as a period of erosion following oil deposition on the beach would lead to the rapid breakdown and dispersal of that oil. If oil is stranded shortly before the onset of an accretion phase, that oil would be buried and would remain so until exposed by a subsequent period of erosion (Fig. 9) (Owens 1977a).

Similar erosion–accretion changes can result from the formation of beach cusps or from the alongshore migration of rhythmic beach topography. These topographic beach features can cause local erosion or burial of oil stranded in the intertidal zone.

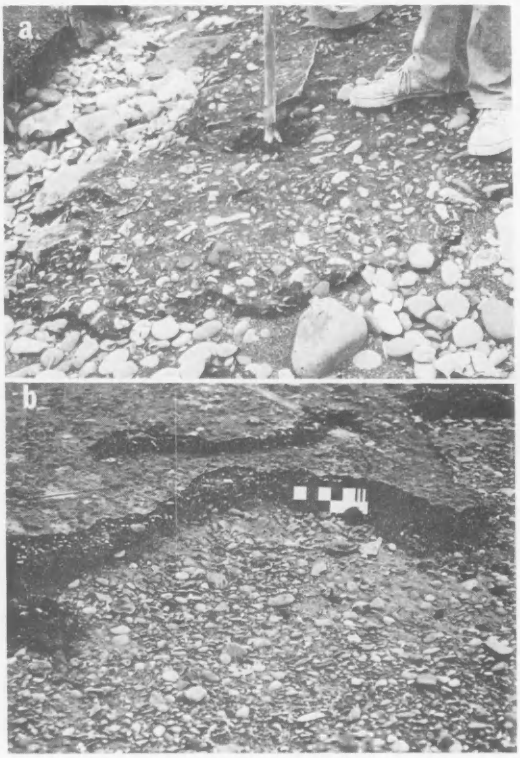



FIG. 7. a, Part of an "asphalt pavement" from an exposed high wave-energy environment, Crichton Island, N.S., 3 yr after the *Arrow* spill (photo by J. R. Belanger, Bedford Institute): b, Erosion along the edge of an oil–sediment "asphalt pavement" in a low wave-energy environment, south shore, Straits of Magellan, 2½ yr after the *Metula* spill.

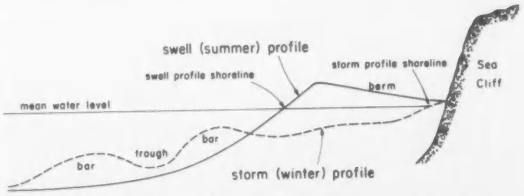



FIG. 8. Idealized summer–winter beach profile changes characteristic of swell-dominated environments such as the west coast of North America (after Komar 1976).

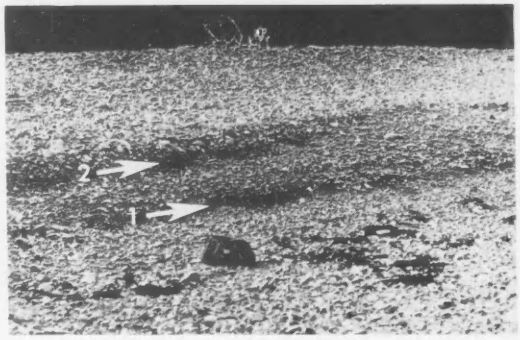



FIG. 9. Buried oil exposed in the beach after a period of erosion, Hadleyville, N.S., August 1970. The fact that two layers are exposed indicates that oil was stranded on two occasions that were separated by a period of beach accretion.

The effect of wave action on the persistence of oil is, therefore, twofold: (1) waves physically break down oil as mechanical energy is dissipated on the shoreline, and as levels of wave energy increase, so rates of physical breakdown and dispersal increase; (2) wave action causes the redistribution of sediments and oil, leading to either burial or erosion of stranded oil.

Superimposed on the spatial and temporal variations in wave-energy levels is the role of tides. The effectiveness of wave action decreases as the tidal range increases because wave energy is dissipated over an increasingly larger vertical range. Therefore, the rates of mechanical dispersal of stranded oil would be higher in a microtidal environment (range $<2$ m) when compared with a macrotidal environment (range $>4$ m) with the same levels of incoming wave energy.

Although the major role of tides is to control the concentration of wave energy across a beach, tidal currents can redistribute intertidal sediments and can therefore abrade, bury, erode, or disperse oil stranded in intertidal areas.

The effect of wind action on the persistence of stranded oil is primarily associated with rates of evaporation and a loss of light fractions from exposed surfaces (McMinn and Golden 1973). In addition, wind-transported fine-grained sediments can bury oil stranded on the beach. This burial would lead to a reduction in the rates of weathering and aging of the oil.

In cold, temperate, or polar environments the rates of natural weathering and aging and the mechanical dispersal of oil can be considerably reduced. In particular, the annual average energy levels at the shoreline are lowered owing to the prevention of wave generation or to the dampening of existing waves by the presence of sea ice. Ice also forms on the shoreline by the freezing of swash or wave spray, and this beach-fast ice (usually referred to as an "ice foot") prevents waves from acting directly on beach sediments or stranded oil. The ice foot usually forms before and persists longer than the sea ice and effectively encloses or buries stranded oil until it is exposed by ice melt during the spring–summer season.

The effects of coastal processes on stranded oil also involve consideration of the feedback effects of the oil itself. This is particularly important when a large volume of oil is deposited on the shoreline. Oil can act as a protective cover and can bind sediments together, thereby preventing sediment redistribution by wave action (Fig. 7). The development of an immobilized beach ("asphalt pavement") is critical in low-energy environ-

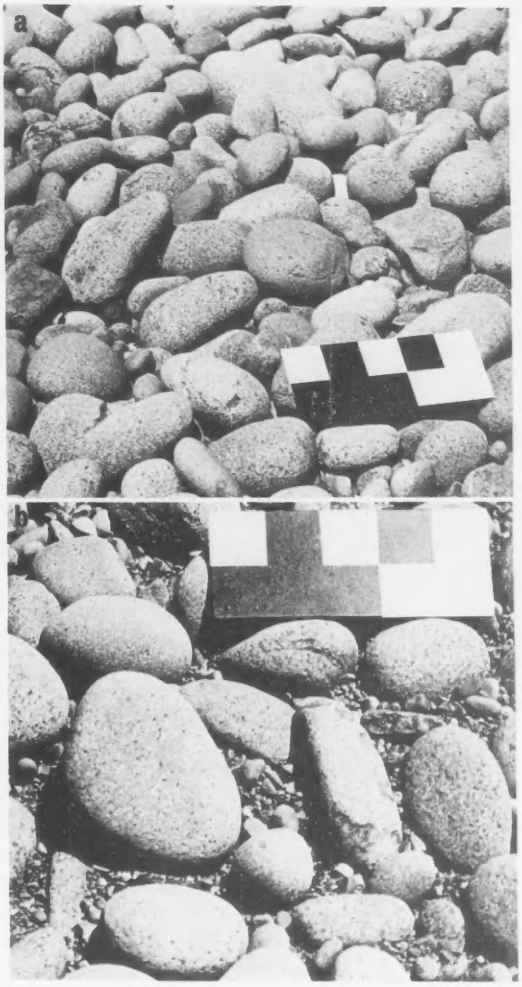



FIG. 10. a, Cobble beach with no sand or pebble fraction and large, open interstitial spaces (scale is 25 cm long). b, Mixed sediments of cobble with sand, granules, and pebbles; the fine fractions fill the void spaces.

ments. Breakdown and dispersal of the oil then occurs only very slowly by the erosion and abrasion of the edges of the oil cover (Fig. 7b). A secondary feedback effect in this instance is that waves cannot use immobilized sediments as an abrasive tool to break down or erode the oil cover.

## Shoreline Factors and the Persistence of Stranded Oil

The effects of the dissipation of available mechanical energy on the shoreline depend on the physical nature of the littoral zone. For this discussion the important shoreline factors are related primarily to the rock type and the character of sediments in the littoral zone. On

FIG. 11. Ice foot at low tide after breakup on an arctic beach, Cape Ricketts, Devon Island, N.W.T. The ice foot is approximately 4-m high and the high-tide line is clearly visible above the person's head.

rock coasts rates of erosion are dependent upon the resistance of the outcrops to hydraulic action, abrasion, or corrosion by wave activity. The persistence of oil stranded on rock exposures is not affected by the resistance of the rocks, and dispersal is a direct function of wave-energy levels.

Where sediments are present in the littoral zone the depth of oil penetration into the interstitial spaces becomes a significant factor in rates of degradation. The depth and the rate of penetration depend on the viscosity and volume of the oil and on the porosity of the sediments. On fine-grained sand beaches, low-viscosity oils will penetrate the small interstitial spaces between particles, but as the time factor increases, even viscous oils can slowly penetrate into the subsurface sediments. On coarse-sediment beaches in which the interstitial spaces are large (Fig. 10a), even heavy, tarry oils can rapidly penetrate down to the groundwater table. Abrasion and dispersal of oil decreases with depth as the mechanical energy from waves is largely expended by the redistribution of surface sediments. It would be expected that oil on the surface of a sand beach, where penetration depths are minimal, would therefore be dispersed more rapidly than on cobble beaches, where oil is distributed through a greater vertical sequence of sediments. In addition, on sand beaches the swash from a breaking wave runs back down the beach face under the influence of gravity, as relatively little water percolates into the subsurface sediments. As a result, energy is available to abrade and disperse stranded oil from swash and backward action. On cobble beaches, however, there is frequently no backwash, as the swash percolates rapidly through the highly porous sediments. In this instance the total available energy is less for the same-sized breaking wave, as the backwash component is largely absent, so that rates of abrasion and dispersion are reduced when compared with the same processes on sand beaches.

Although these factors would indicate that for the same level of incoming wave energy sand beaches would

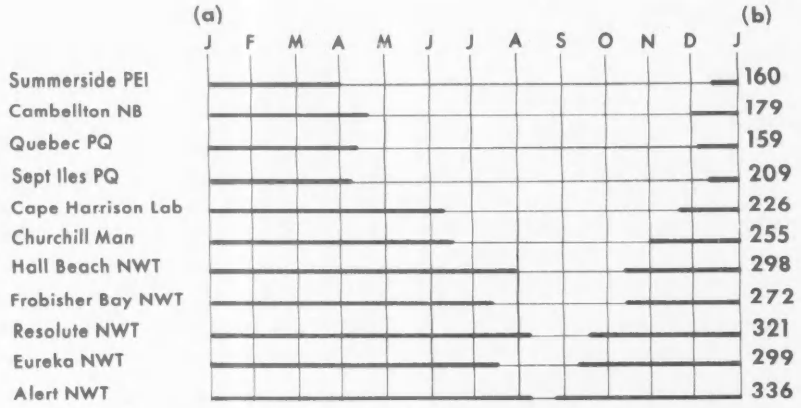



FIG. 12. a, Duration of ice on beaches or in the nearshore zone during average years (data from Allen 1964). b, Number of days per year with frost (from Environment Canada 1973).

be cleaned faster than cobble beaches, it must be added that well-sorted cobble sediments tend to accumulate only in high wave-energy environments and in these conditions persistence times would be low. In lower energy environments sediment sorting is less well developed and the interstitial spaces between cobbles contain sand- and granule-sized materials that reduce the porosity of the beach (Fig. 10b).

## Climate and Mechanical Energy Levels

Wave-energy levels vary throughout the world as a function of two factors: (1) wind velocity, direction, and duration; and (2) the fetch area over which waves can be generated. Climate plays a critical role in reducing fetch areas in high latitudes owing to the presence of sea ice. The primary effect of the reduction in ambient temperatures in polar regions is to increase the distribution of sea ice and thereby reduce the potential for wave generation. As mentioned above, ice also forms on the beach itself as an ice foot (Fig. 11) and can prevent surface wind-generated waves from dissipating their energy on the shoreline and abrading stranded oil. The formation of an ice foot is a function of ambient air and water temperatures.

The average duration of ice on the beach or in the nearshore zone for eastern Canada is shown in Fig. 12. It is evident from this diagram that independent of the ability of winds to generate surface waves and independent of the fetch area, the time available for the mechanical abrasion and dispersion of stranded oil decreases as a function of the increase in the distribution of ice. In northern arctic areas wave generation is limited to only a few months each year (McCann 1973). High levels of wave activity in these arctic coastal environments are infrequent owing to the requirements that the sea over which the waves are generated be ice free and that the wind direction coincide with the open-water fetch for a particular shoreline during a long-enough period to generate high waves. In the Gulf of St. Lawrence, an area of locally generated waves, maximum wind velocities occur during October to March. In this region sea ice limits wave generation from December to April and therefore considerably reduces levels of wave energy during winter, at a time when they would otherwise be at a maximum (Owens 1976).

In latitudes where open water is present for only a few months each year, littoral processes are greatly modified. Rates of longshore sediment transport are low, and the beach is rarely subject to changes, such as storm-wave erosion or migrating rhythmic features. As a result, erosion or burial of oil would occur only during the infrequent periods of wave activity. In parts of the Queen Elizabeth Islands, periods of storm waves could be as infrequent as every 2 or 3 yr.

During periods when the beach is free of ice, individual small or large floes can be pushed into the littoral zone by wind action. If the ice grounds on the beach, it can push sediment landward to form a ridge (Owens and McCann 1970). This would affect the oil on the beach by mixing it with the beach sediments or by burying it beneath the ice-push ridge. An important effect of ice pushing sediments and oil up the beach would be that the oil would be deposited above the maximum limit of wave activity. Rates of degradation would then be lowered, as the oil would be buried and would not be subject to reworking and dispersion by littoral processes.

On beaches in arctic and subarctic environments, water in the sediments freezes during periods of subzero temperatures. This subsurface ice fills the spaces between sediment particles and acts as a lower limit for

oil penetration. In the arctic archipelago the depth to the "frost table" (i.e. the depth to which the beach thaws and is ice free) varies from <25 cm in winter and spring to a maximum of ~100 cm in summer and autumn (Owens and McCann 1970; Taylor and McCann 1974; Owens and Harper 1977). Oil on a beach in early summer could penetrate to greater depths as the frost table melts. Oil that penetrates a beach during the arctic summer could become enclosed as the frost-table depth decreases in autumn and winter. This enclosed oil could then be released the following summer.

The persistence of stranded oil in cold environments is also further affected by an increase in viscosity and a possible decrease in the rates of microbial and chemical degradation at the lower temperatures. However, as noted earlier, the importance of mechanical energy levels in the dispersal of stranded oil is of greater importance than in situ weathering when consideration is given to the removal of stranded oil from the coast.

## Conclusion

There exists a basic relationship between levels of mechanical energy at the shoreline and the persistence of oil stranded in the littoral zone that is largely independent of the type of oil. It is necessary when estimating rates of mechanical dispersal to take into account the regional character of the wave climate and the local exposure (or sheltering) of a particular section of shoreline. Within this generalized concept, consideration must also be given to (1) the timing of the beach cycles, (2) the depth of penetration of the oil, and (3) the effects of creation of an "asphalt pavement" by large volumes of oil.

In colder climates as a corollary to the relationship between mechanical energy levels and the persistence of stranded oil, there is an interdependence between mechanical energy levels and temperature. These are in turn a function of beach and sea-ice development. In cold climates the possible reduction in the rates of thermal, biological, and chemical energy inputs further results in the increasing importance of mechanical energy levels, which are themselves also reduced.

With basic information on coastal processes and shoreline factors it is possible to estimate approximate rates of the dispersal and residence times of stranded oil in the littoral zone. This knowledge is of critical importance in decisions related to cleanup programs. In high wave-energy environments that have a rapid self-cleaning capability, restoration may be an unnecessary operation. However, heavy oils stranded in a low-energy environment, particularly in cold or arctic regions, would be expected to persist for tens of years with little loss of volume. In general, therefore, as energy levels at the shoreline increase, recovery rates will also increase and the subsequent requirements for a cleanup operation or restoration program will decrease.

## Acknowledgments

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# Microbial Ecology Studies of the *Metula* Spill in the Straits of Magellan[1]

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COLWELL, R. R., A. L. MILLS, J. D. WALKER, P. GARCIA-TELLO, AND V. CAMPOS-P. 1978. Microbial ecology studies of the *Metula* spill in the Straits of Magellan. J. Fish. Res. Board Can. 35: 573–580.

The grounding of the tanker V.L.C.C. *Metula* in the Straits of Magellan in August 1974 provided a unique opportunity to study a massive spill in a cold region on a long-term basis. Removal of spilled oil from the *Metula* was solely by in situ physiochemical and biological mechanisms. Microbiological studies undertaken in May 1976 showed increased heterotrophic bacterial populations at oil-impacted sites. A cold-tolerant population of petroleum degrading bacteria was observed. From biodegradation studies, we concluded that oil degradation under in situ conditions proceeds relatively slowly, with marked persistence of *Metula* oil in the Straits of Magellan 2 yr after the original spill.

*Key words:* petroleum degradation, oil spills, Straits of Magellan, microbial degradation of oil, *Metula*

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L'échouage du pétrolier V.L.C.C. *Metula* dans le détroit de Magellan en août 1974 fournit une occasion unique d'étudier les effets à long terme d'un déversement massif dans une région froide. L'enlèvement du pétrole déversé par le *Metula* se fit exclusivement par des moyens physico-chimiques et biologiques in situ. Des études microbiologiques entreprises en mai 1976 indiquent la présence de populations bactériennes hétérotrophes accrues aux endroits affectés par le pétrole. On a observé une population tolérante du froid de bactéries capables de dégrader le pétrole. Des études de biodégradation nous permettent de conclure que la dégradation du pétrole dans des conditions *in situ* se poursuit à un rythme relativement lent, avec persistance marquée de pétrole du *Metula* dans le détroit de Magellan 3 ans après le déversement.

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INCREASED oil spillage in the colder regions of the earth will be a result of petroleum exploration and development. One such event has already occurred, the grounding of the tanker V.L.C.C. *Metula* in the Straits of Magellan in August 1974. Although it was an ecological mishap, the *Metula* incident did provide a unique opportunity to study a massive spill in a cold region on a long-term basis. No effort was made at any time during or after the spill to conduct a cleanup operation. Thus, removal of spilled oil was solely by in situ physicochemical and biological mechanisms. Even more interesting is the fact that data gathered on the *Metula* spill can be extrapolated directly to similar events that might occur in the coastal regions of the northeastern and northwestern United States. A survey of the geomorphology of the beaches in the Straits area (Hayes et al., personal communication, University of South Carolina, Columbia, S.C.) revealed a striking correspondence between the eastern portion of the Straits and the south coast of Alaska, particularly the Cook Inlet area. Similarly, the western portion of the Straits are similar in geomorphology to portions of the northeastern coast of the United States, including parts of New Hampshire, Massachusetts, and Long Island, New York. Both Alaska and the New England regions are either pro-

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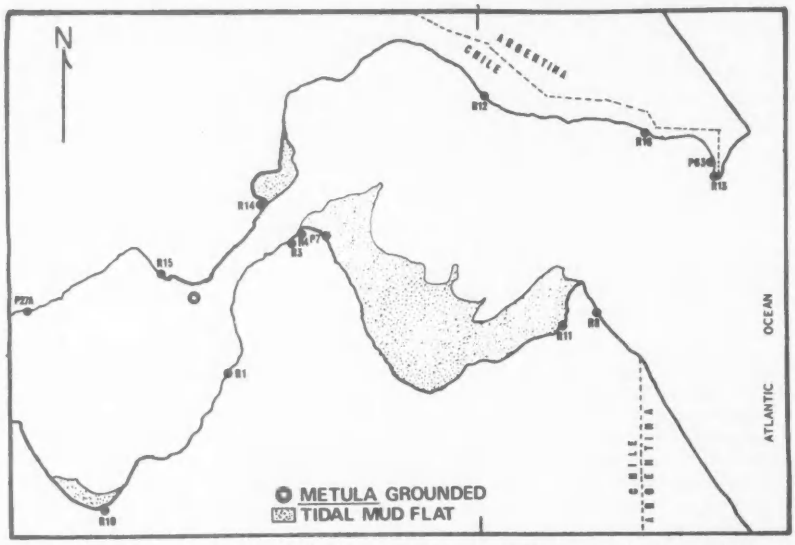


FIG. 1. Location of stations in the Straits of Magellan, Tierra del Fuego, included in the study.

posed or scheduled for increased petroleum traffic in the near future.

The *Metula* ran aground at the west end of the first narrows (Fig. 1) on August 9, 1974. However, the spilling of oil was not controlled until September 15, by which time 51 500 tons of oil were lost.

The location of the *Metula* oil spill is in a region classified as a modified middle latitude steppe, i.e. an area having 250–500 mm of precipitation/yr, with cold winters. However, the temperature in this region is modified by the surrounding water masses, and mean morning winter temperatures do not go below 0°C. No temperature data are available for the *Metula* impact area, but the temperature data for nearby Punta Arenas are indicative of the temperatures for the Straits. Mean summer temperatures in Punta Arenas range between 8 and 11°C, and mean winter temperatures between 1 and 4°C. Mean monthly maxima and minima vary much more than the average seasonal temperature would suggest.

The research efforts of the project reported here were focused on seeking answers to two major points: (1) the effect of the spill on the indigenous, i.e. autochthonous, microflora of the beaches; and (2) the biodegradative potential of the microflora.

## Materials and Methods

To assess the impact of the spill on the beach microflora, several physiological types of organisms were enumerated as follows: total viable, aerobic, heterotrophic microorganisms (assessed according to Walker and Colwell 1973, 1976); petroleum-degrading microorganisms (enumerated by MPN method of Mills et al. 1978); starch-hydrolyzing microorganisms; chitin-digesting microorganisms; casein degraders; Tween 20 degraders; cellulose degraders; agar digesters; yeasts; and filamentous fungi. A total of 14 stations was sampled including sites where heavy oil contamination occurred, as well as areas free of oil as determined visually and by chemical (gas–liquid chromatography) analysis (Fig. 1). The grounding of the *Metula* occurred in August 1974 and the initial sampling was accomplished in May 1976. Thus, immediate effects of the spill were not determined. However, a team of investigators visited the Straits in January 1975. They collected several samples of oiled sand for our laboratory, and preliminary analyses were done, the results of which are published elsewhere (Colwell et al. 1978). In addition, several litres of oil taken from the cargo tanks of the *Metula* shortly after grounding were made available for our studies. To simulate the effect of the oil spill on the beaches, during the initial visit to the area in May 1976, a litre of *Metula* oil was "spilled" on the sand at two beach sites: an oil-free area and an area contaminated by the original *Metula* spill. Microbiological analysis of samples from the spills were compared with samples collected immediately adjacent to the spill sites.

Total viable aerobic heterotrophs in the "minispill" and in the oil-free samples, collected at the station uncontaminated by the *Metula* spill, were enumerated (Table 1). Samples were collected in January 1977, by which time the stakes used to mark the boundaries of the oil amendment had been covered by sand. Thus, it was necessary to dig deeply into the sand to reach the spot where the "spill" had been created.

Two sand samples were amended with oil to final concentrations of 1%, using *Metula* oil. One of the samples was collected at an oil-free area at San Gregorio, Chile, in the Straits of Magellan, the other from the shore of the Rhode River, a subestuary of Chesapeake Bay. Samples collected from the sites were incubated for 1 mo at 22 and 3°C, i.e. for the Chesapeake Bay and Straits of Magellan

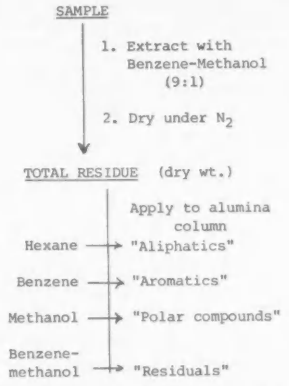


FIG. 2. Procedure followed for determining the extent of biodegradation of *Metula* oil by mixed cultures of microorganisms present in San Gregorio beach sand and Rhode River sediment.

samples, respectively. At the end of the incubation period, dilutions of the sediment samples were inoculated into flasks containing a sterile marine-strength salts solution (Walker and Colwell 1973), to which was added 0.1% $NH_4NO_3$ and 1.0% (vol/vol) *Metula* oil. The flasks were part of a gas-train apparatus similar to that described by Atlas and Bartha (1972), designed to scrub $CO_2$ from the influent air so that effluent $CO_2$ is trapped in an alkaline solution and titrated against an acid. The amount of $CO_2$ produced by microorganisms growing on oil is a direct measurement of mineralization of the petroleum hydrocarbons. The incubations were carried out at both 22 and 3°C for each of the temperature-acclimated samples.

At the end of the incubation period, the extent of degradation of the oil was determined (Fig. 2). The contents of each flask were extracted with benzene–methanol (9:1) and dried to a constant weight under a stream of high purity-dry $N_2$. The resultant weight represented the undegraded material. After weighing, each of the samples was separated into fractions by chromatography on alumina columns. Each of the resulting fractions was dried under $N_2$, and weighed.

Losses in amount of total extractable materials in the flasks after incubation were determined (Table 6). Percent degradation was calculated by dividing the amount of recoverable material in the experimental flasks by the amount remaining in an uninoculated flask for each treatment.

## Results and Discussion

With the exception of the January 1977 sampling, an increase in number of bacteria was observed in the spill area compared with the oil-free site. It was difficult, because of the shifting of the sand, to know whether the apparent return to normal, i.e. lower heterotrophic counts, observed in January 1977 was genuine or an aberrance arising from the fact that the collection of the sample may not have been precisely where the added oil was located.

An increase in numbers of heterotrophic microorganisms was observed in samples of oil-amended sand at the station under study that had previously been polluted with oil. However, the stimulatory effect of oil was not as dramatic for those samples collected at that station as was observed for similar samples collected at the station free of *Metula* oil. This was due in part, perhaps, to higher populations of heterotrophs observed to be present at the contaminated site (Table 1).

Thus, oil contamination of a beach was found to be associated with an immediate and dramatic increase in the numbers of aerobic heterotrophic microorganisms, with increased numbers of heterotrophs persisting for a significant period of time.

In Table 2 a comparison of heterotrophic counts for samples taken at several different locations in the Straits of Magellan is provided. As previously stated, samples were collected from areas where oil from the *Metula* spill was still in evidence, as well as from areas where no oil could be observed. A further comparison was made to determine the effect of sediment texture on the counts recovered from the samples. From the results obtained in this study, there was clearly a significant increase in the heterotrophic counts for oil-containing samples, compared with samples free of oil. Analysis of variance showed the difference to be significant at the 99.9% level. No significance, however, could be attributed to differences in texture of the sediment.

Similar analyses were performed on petroleum degraders (Table 3). In the experimental "minispill" carried out at the oil-free station, numbers of petroleum degraders showed a significant increase compared with adjacent, uncontaminated sand. The increase in petroleum degraders persisted throughout the sampling, even into January 1977. At the station impacted by oil from the *Metula* tanker spill, addition of fresh oil caused only a slight increase in the number of petroleum degraders, and this increase was observed only for the first set of samples collected in June, and not in subsequent samples.

Results of analyses of the distribution of petroleum

TABLE 1. Total, viable, aerobic heterotrophic microorganisms enumerated at stations 11 and 27, measured as colony-forming units per millilitre. Station 11 was impacted by the *Metula* spill; station 27 was unaffected.

| Dates visited | Sample type | |
|---|---|---|
| | Unamended | Oil added |
| | *Station 11* | |
| May 20, 1976 | $1.5\times10^{6}$ | — |
| June 6, 1976 | $2.5\times10^{7}$ | $7.0\times10^{7}$ |
| June 19, 1976 | $1.5\times10^{7}$ | $6.4\times10^{7}$ |
| Aug. 16, 1976 | $6.9\times10^{7}$ | $1.6\times10^{8}$ |
| | *Station 27* | |
| May 18, 1976 | $2.4\times10^{7}$ | — |
| June 3, 1976 | $1.4\times10^{7}$ | $1.5\times10^{8}$ |
| June 19, 1976 | $1.5\times10^{6}$ | $2.1\times10^{7}$ |
| Aug. 15, 1976 | $8.9\times10^{6}$ | $2.5\times10^{8}$ |
| Jan. 25, 1977 | $9.8\times10^{5}$ | $4.9\times10^{5}$ |

TABLE 2. Comparison of numbers of total viable heterotrophic microorganisms in oiled and unoiled beach sands, measured as colony-forming units per millilitre. Number in parentheses indicates number of samples analyzed.

| Texture | Condition | |
|---|---|---|
| | No oil added | Oil added |
| Sand | $9.8\times10^{2}$ (40) | $4.5\times10^{3}$ (48) |
| Fine sand | $6.1\times10^{2}$ (32) | $3.9\times10^{3}$ (34) |

TABLE 3. Most probable number (in colony-forming units) per millilitre of sand slurry (MPN) of petroleum degraders detected in the beach sand samples collected at stations 11 and 27. Station 11 was impacted by the *Metula* spill; station 27 was unaffected.

| Dates visited | Sample type | |
|---|---|---|
| | Unamended | Oil added |
| | *Station 11* | |
| May 20, 1976 | $1.8\times10^{2}$ | — |
| June 6, 1976 | $2.6\times10^{1}$ | $7.9\times10^{1}$ |
| June 19, 1976 | $7.9\times10^{1}$ | $4.9\times10^{1}$ |
| Aug. 16, 1976 | $2.5\times10^{1}$ | $2.4\times10^{1}$ |
| | *Station 27* | |
| May 18, 1976 | $7.8\times10^{-1}$ | — |
| June 3, 1976 | $7.0\times10^{1}$ | $1.6\times10^{4}$ |
| June 19, 1976 | $1.3\times10^{1}$ | $7.0\times10^{1}$ |
| Aug. 13, 1976 | $1.1\times10^{1}$ | $2.1\times10^{1}$ |
| Jan. 25, 1977 | $1.7\times10^{2}$ | $3.5\times10^{3}$ |

TABLE 4. Comparison of numbers of petroleum degraders in oiled and unoiled beach sand samples, measured as colony-forming units per millilitre. Numbers in parentheses represent number of samples examined.

| Texture | Condition | |
|---|---|---|
| | No oil | Oil |
| Sand | $1.9\times10^{1}$ (12) | $8.7\times10^{3}$ (18) |
| Fine sand | $4.1\times10^{1}$ (3) | $7.1\times10^{3}$ (7) |

degraders, with respect to texture of the substratum and presence or absence of oil, are given in Table 4. As was observed for the total aerobic heterotrophic microorganisms, oil was found to be associated with an increase in numbers of petroleum degraders, but no correlation with sediment texture was detected.

The stimulatory effect of oil on the microbial populations was also observed when other physiological groups of microorganisms were examined. However, when the proportional representation of a given physiological group in the total heterotrophic population was determined, a different kind of impact of the oil was discovered. That is, starch hydrolyzers, chitin digesters, and petroleum degraders, and the respective percentage of these physiological groups with reference to the total viable count for oiled and unoiled samples, were affected by the oil (Table 5). Starch hydrolyzers and chitin digesters contributed less to the total viable population in the presence of oil proportionally. Because the numbers of organisms recovered from oil-impacted samples were larger, we conclude that there can be a stimulatory effect of oil on the total heterotrophic microbial population. The decrease in percentage composition of the population, however, indicates differential stimulation with respect to the various physiological groups that make up the population. That is, enhancement of growth for one or more physiological groups over that observed for other physiological groups was observed, a common phenomenon when enrichment is accomplished.

Oil was found to cause a dramatic increase in numbers of petroleum degraders (Table 5). However, proportionately, only a small increase in percentage of heterotrophs that were oil degraders was found, and the significance of the difference is negligible due to the large variance in the calculated percentages, as indicated by the standard deviation. Although a radical population shift toward petroleum degraders could be expected, such a shift was not observed. Percentage increase or decrease in proportional representation of other microorganisms enumerated were either inconsequential or indeterminable due to large variances.

Several investigators have reported toxic effects of oil on microorganisms (Young and Mitchell 1973; Shindler et al. 1975; Walker et al. 1975; Walker and Colwell 1975). Hodson et al. (1977), using radiolabeled-glucose uptake and metabolism as indicators of microbial activity, showed that Louisiana crude oil, Kuwait crude oil, No. 2 fuel oil, and Bunker C fuel oil were toxic to marine organisms to a varying extent. The processed oils, No. 2 fuel oil and Bunker C, were more toxic than either of the two crude oils and Kuwait crude was ob-

TABLE 5. Effect of *Metula* oil on representation of physiological groups of microorganisms comprising the heterotrophic population of beach sand, given as percent of the total viable count (TVC). SD = standard deviation.

| Physiological group | Clean sand | | | Oil-impacted sand | | |
|---|---|---|---|---|---|---|
| | Mean % of TVC | No. of samples examined | SD | Mean % of TVC | No. of samples examined | SD |
| Starch | 17.1 | 9 | 8.7 | 3.7 | 13 | 6.2 |
| Chitin | 6.6 | 19 | 8.1 | 1.8 | 15 | 1.6 |
| Petroleum | .018 | 17 | 0.043 | 0.050 | 19 | 0.145 |

served to be the least toxic. In the studies reported here, *Metula* oil, a light Arabian crude similar to Kuwait crude, demonstrated little if any effect on glucose metabolism in microorganisms present in oil-free and in oiled sands.

In addition to the studies on effects of *Metula* oil on the beach microflora, experiments were carried out to determine the fate of oil deposited on beach sand and the role, if any, of microorganisms in degradation of the oil. Low temperatures have been found by several investigators to reduce the rate of microbial utilization of hydrocarbons. Utilization does occur, however, at low temperatures, including arctic temperatures. Nevertheless, to determine whether or not a cold-tolerant population of petroleum-degrading microorganisms was present in the sands of the Straits of Magellan, a series of experiments was carried out.

Evolved $CO_2$ was measured at intervals during incubation. In flasks containing 1.0% *Metula* oil, mineralization at 22°C proceeded similarly for 3°C- and 22°C-acclimated sand samples. At 3°C, some difference in growth and mineralization was noted and the 3°C-acclimated sand sample collected from San Gregorio, Chile, showed greater mineralization of the oil (Fig. 3). Results obtained when *Metula* oil was employed at a concentration of 0.1% showed little difference between samples acclimated to the temperatures indicated (Fig. 4). Thus, a cold-tolerant population capable of rapid mineralization of hydrocarbons at low temperature may occur in the Straits of Magellan, but the differences in the capabilities of that population and populations of microorganisms from a more temperate climate but acclimated to the low temperature are not extremely great.

Degradation occurring in flasks incubated at 22°C showed little difference in amount of degradation from that of either the 3°C- or 22°C-acclimated sand samples (Table 6). However, degradation occurring in samples at 3°C showed that a larger percentage of the oil was degraded, in the case of the Chilean beach sand sample preincubated at 3°C, than the sample preincubated at 22°C, indicating the presence of a cold-tolerant petroleum-degrading population.

The recovery of aliphatics from weathered and degraded *Metula* oil is given in Table 7. In all of the uninoculated treatments, the amount of material recovered was 103–108% of the added amount (319 mg in the 1% and 31.9 mg in the 0.1% flasks). In the presence of an inoculum, a large decrease in aliphatic material present was observed. At 22°C, both populations of microorganisms degraded significant portions of the *Metula* oil. When the concentration of oil was at 1%, little difference was noted. However, when 0.1% oil was used, the microbial population acclimated to 22°C

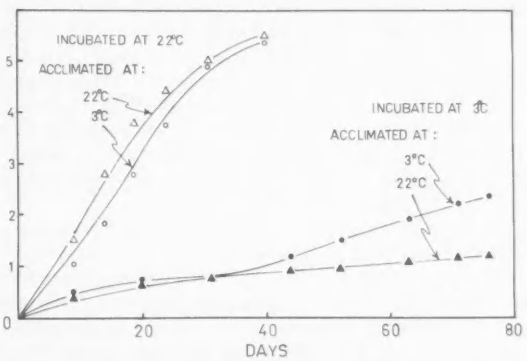


FIG. 3. Mineralization of 1% *Metula* crude oil at 3 and 22°C by acclimated populations of microorganisms in San Gregorio beach sand and Rhode River sediment samples.

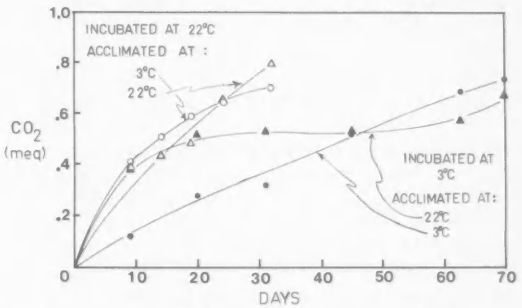


FIG. 4. Mineralization of 0.1% *Metula* crude oil at 3 and 22°C by acclimated populations of microorganisms in San Gregorio beach sand and Rhode River sediment samples.

TABLE 6. Degradation of *Metula* crude oil incubated at 3 and 22°C and inoculated with mixed cultures in beach sand samples acclimated at 3 and 22°C.

| Amount of oil added (%) | Incubation temp (°C) | Acclimation temp (°C) | % oil degraded |
|---|---|---|---|
| 1 | 22 | 22 | 26.9 |
| | | 3 | 22.3 |
| 0.1 | 22 | 22 | 21.3 |
| | | 3 | 20.2 |
| 1 | 3 | 22 | 10.4 |
| | | 3 | 21.3 |
| 0.1 | 3 | 22 | 18.9 |
| | | 3 | 48.4 |

TABLE 7. Degradation and weathering of the aliphatic fraction of *Metula* oil at 22 and 3°C by beach sands acclimated to 22 and 3°C[a]. Values are expressed as milligrams recovered.

| | Incubation temp | | | |
|---|---|---|---|---|
| | 22°C | | 3°C | |
| | Concn of oil (vol/vol) | | | |
| Treatment | 1% | 0.1% | 1% | 0.1% |
| Uninoculated | 327 | 34.3 | 338 | 34.3 |
| Acclimated at 22°C | 180 | 12.4 | 292 | 20.1 |
| Acclimated at 3°C | 185 | 23.4 | 258 | 11.6 |

[a]*Metula* oil contains 319 mg of analyzable aliphatics/mL oil.

TABLE 8. Degradation and weathering of the aromatic fraction of *Metula* oil by microbial populations incubated at 22 and 3°C[a]. Values are expressed as milligrams recovered.

| | Incubation temp | | | |
|---|---|---|---|---|
| | 22°C | | 3°C | |
| | Concn of oil (vol/vol) | | | |
| Treatment | 1% | 0.1% | 1% | 0.1% |
| Uninoculated | 130 | 16.3 | 88.9 | 12.6 |
| 22°C enrichment | 127 | 12.2 | 155 | 12.5 |
| 3°C enrichment | 141 | 15.1 | 103 | 9.05 |

[a]*Metula* oil contains 207 mg of analyzable aromatics/mL oil.

TABLE 9. Effect of incubation on the polar fraction of *Metula* oil. The *Metula* oil contained 27 mg of recoverable polar compounds/mL.

| | Incubation temp | | | |
|---|---|---|---|---|
| | 22°C | | 3°C | |
| | Concn of oil (vol/vol) | | | |
| Treatment | 1% | 0.1% | 1% | 0.1% |
| Uninoculated | 46.8 | 5.1 | 109.4 | 9.9 |
| 22°C enriched | 36.5 | 9.5 | 80.2 | 15.6 |
| 3°C enriched | 50.6 | 5.7 | 83.0 | 12.7 |

was more efficient in utilizing aliphatic compounds. When the incubation temperature was 3°C, both populations were relatively efficient in degradation of the aliphatic fraction of the *Metula* oil. However, the sample inoculated into the petroleum medium and acclimated at 3°C was significantly more effective in degradation of the aliphatic fraction.

Biodegradation of the aromatic fraction of the *Metula* oil is indicated in Table 8. Weathering of the compounds in this fraction appeared to be the major mode of removal or alteration. Extracts from flasks inoculated with either of the two temperature acclimatized populations showed little or no loss of aromatics in excess of that in the uninoculated flasks, except that at 3°C, the microbial populations degraded some of the aromatics in excess of degradation accomplished by the microbial populations acclimated to 22°C. The apparent large increase in aromatics in inoculated flasks incubated at 3°C and containing 1% oil was probably due to analytical error arising during fractionation of the uninoculated sample, i.e. incomplete elution of the aromatic fraction, explaining not only the low value obtained for the aromatic fraction, but also the high value for polar compounds obtained for that same treatment (Table 9).

Changes in the fraction containing polar compounds are given in Table 9. In all cases, inoculation of *Metula* oil with beach sand or sediment resulted in net production of polar material, in comparison with the uninoculated *Metula* oil, regardless of the presence or absence of inoculum, incubation temperature, or the concentration of substrate used. However, in general, inoculation caused an increase in polar material in excess of that caused by weathering.

The residual fraction was reduced in all cases, compared with the uninoculated, nonweathered *Metula* oil (Table 10). The primary cause of the reduction appeared to be weathering, because a large portion of the total loss was observed in the uninoculated flasks.

Gas-chromatographic analysis of the aliphatic fractions revealed no differences among the various treatments. Inoculated samples were always different from weathered samples in that the major peaks representing the *n*-alkanes were absent from the inoculated extracts. The unresolved "envelope" was present in both weathered and degraded samples.

The results presented here indicate that in a situation ideal for biodegradation occurring over a period of time, most of the aliphatic material will be weathered and/or degraded and the remaining oil will comprise large amounts of fractions of oil other than aliphatics. The rate at which the degradation of each of the fractions occurs depends, of course, on nutrients available in the given ecosystem, specifically nitrogen and phosphorus in the case of the coarse sediment material, or

TABLE 10. Effect of weathering and microbial activity on the residual fraction of *Metula* oil. The fresh oil contained 96 mg of residual material/mL.

| Treatment | Incubation temp: 22°C, Concn of oil (vol/vol) 1% | 22°C 0.1% | 3°C 1% | 3°C 0.1% |
|---|---|---|---|---|
| Uninoculated | 36.6 | 2.0 | 48.5 | 6.0 |
| 22°C enrichment | 28.3 | 6.2 | 39.3 | 5.1 |
| 3°C enrichment | 35.6 | 4.0 | 36.3 | 2.2 |

TABLE 11. Fractional composition of extracted samples from the Straits of Magellan.

| Sample | Fractional percentage: Aliphatic | Aromatic | Polar | Residual |
|---|---|---|---|---|
| Asphalt Banco Lomas | 43.1 | 19.2 | 1.4 | 17.4 |
| Asphalt R 1 | 46.2 | 24.3 | 1.5 | 9.4 |
| Asphalt-sand R 3 | 28.9 | 13.9 | 4.5 | 44.0 |
| Tar ball R 8 | 46.4 | 32.7 | 12.2 | 11.4 |

$O_2$, in the finer sands, as shown by Gibbs and Davis (1976).

Extraction and analysis of petroleum residues from the beach sand samples collected in 1976 from the Straits of Magellan showed that the system in which biodegradation was still in progress after the 1974 spill was not ideal for rapid and complete degradation. An area of beach that was massively impacted by oil during the *Metula* spill indicates that after 1½ yr the oil remaining on the beaches has assumed the appearance of a soft asphalt highway. In fact, the petroleum at some of the heavily impacted sites has formed an aggregation of rocks, sand, and other beach material with the oil.

Results of chemical analyses of the asphalt and tarball material suggest that large amounts of aliphatics still are detectable in three of the four samples, suggesting that inside the tar ball or oil aggregates on the beaches, there is largely undegraded oil. On the other hand, the fractional composition of the asphalt–sand mixture found at station R 3 was more weathered and degraded (Table 11).

In summary, general conclusions that can be drawn from this study are as follows:

1) Petroleum contamination by crude oil, such as *Metula* oil, can by selective stimulation induce major shifts in the structure of microbial populations. The ecological significance of these changes can only be inferred until additional experiments are completed. Clearly, however, changes in the population of chitin, cellulose, protein, and other physiological groups of microorganisms will affect the total microbial ecology of an oil-impacted system.

2) Temperature does not seem to be a limiting factor for petroleum degradation in the antarctic marine environment.

3) Oil is degraded slowly by microorganisms in the marine environment, most probably because of limitations imposed by relatively low concentrations of nitrogen and phosphorus available in seawater, as well as restricted accessibility to degradable compounds, especially in aggregated oils or "tar balls."

4) Weathering can be significant, along with microbial degradation, in removal of residues. However, neither weathering nor microbial degradation is effective if the oil is deposited and subsequently buried, or forms asphalt layers or tar ball.

5) Weathering as well as microbial action may in some cases contribute significantly to the formation of polar material.

Because of the low concentrations of nutrients in seawater, along with the inaccessibility of hydrocarbon components inside the asphalt-like concretions and aggregations, tar balls and aggregates will persist on the beaches until wave and/or wind action either mechanically pulverizes them, thereby rendering the oil subject to weathering and degradation, or transports the material out to sea. In conclusion, the impact of the *Metula* spill on the beaches of the Straits of Magellan and Tierra del Fuego was significant and effects are detectable more than 2 yr after the spill. Microbial degradation, weathering, and mechanical action of the waves and surf are slowly removing the residual oil from the *Metula* spill. However, complete removal of the last traces of the *Metula* incident will not occur for years, perhaps decades.

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# Distribution and Abundance of Hydrocarbon-Utilizing Bacteria in Sediments of Chedabucto Bay, Nova Scotia, in 1976[1]

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An extensive reexamination of Chedabucto Bay, Nova Scotia, sediments was carried out in the summer of 1976 for comparison with data obtained shortly after the major oil spill in 1970. Only 2 of the 79 sediment samples examined microbiologically had large numbers of hydrocarbon-utilizing bacteria. The bulk of sediments were, with regard to bacterial populations, comparable to areas described as "clean," i.e. no apparent or known contamination with oil.

*Key words:* Chedabucto Bay, hydrocarbon, hydrocarbon-utilizing bacteria, petroleum, sediment

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Au cours de l'été 1976, nous avons fait un réexamen approfondi des sédiments de la baie Chedabouctou, Nouvelle-Ecosse, dans le but de comparer avec les données recueillies peu après l'important déversement de pétrole en 1970. Seulement 2 des 79 échantillons de sédiments examinés microbiologiquement contenaient de grands nombres de bactéries capables d'utiliser les hydrocarbures. Sous le rapport des populations bactériennes, la majeure partie des sédiments sont comparables à ceux de régions décrites comme « propres », i.e. dépourvues de contamination apparente ou connue par le pétrole.

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THE early experiments of Söhngen (1913) demonstrated definitively that a large array of microorganisms could use hydrocarbons for energy and growth. Since then others have shown that the relevant organisms are widely distributed and show a marked species specificity for the particular hydrocarbons utilized, and that in general no special conditions are required for hydrocarbon utilization.

Concern over the impact of oil in the oceanic environment has raised questions as to the extent to which HCU (hydrocarbon-utilizing microorganisms) are present and their role in cleansing marine areas. A major spill of Bunker C fuel oil in Chedabucto Bay in February 1970 focused attention more specifically on the problem in relation to a relatively severe north temperate marine environment (Anon. 1970).

Part of the answer has been provided by surveys of which the more recent include those of Polyakova (1963) in Neva Bay in the Gulf of Finland; Kinney et al. (1969) in Cook Inlet, Alaska; Mironov (1970) in the Black Sea and Indian Ocean; Gunkel (1973) in the North Sea; Mulkin-Phillips and Stewart (1974a) in the northwestern Atlantic; and Pierce et al. (1975) on an estuarine beach. The general conclusions were that these microorganisms are ubiquitous, occur in greatest numbers in proximity to land, and their numbers in the water column can be a good index of the presence or absence of oil pollutants. Sediment sampling is of particular importance in oil spill areas where oil is entrapped and stationary.

Included among the parallel investigations related to the Chedabucto Bay oil spill in 1970 was microbiological work to determine the proportions of indigenous hydrocarbon-utilizing microorganisms and possible benefits that might accrue from their presence

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(Cundell and Traxler 1973; Mulkins-Phillips and Stewart 1974a, b, c). In 1976 an extensive reexamination of the area was conducted to assess the recovery of the Bay; as part of this study a set of 79 sediment samples was analyzed microbiologically. These samples were frozen immediately with liquid nitrogen and stored frozen for later examination. Validation of this procedure formed a part of the overall analysis.

## Materials and Methods

Validation of the procedure of freezing sediment samples with liquid nitrogen and subsequent storage at temperatures of −15°C to −20°C was conducted on marine sediments and water samples collected in Point Pleasant Park, Halifax. Each sample was homogenized manually and then divided into 35 subsamples. One set of five subsamples was used immediately to determine the total microbial numbers in the starting material; the remainder in sets of five were frozen at the same time with liquid nitrogen and either thawed rapidly immediately after freezing or stored as indicated in the results and then thawed rapidly for analysis. Rapid thawing was achieved by immersing the subsample container in running water maintained at 25°C. Total microbial numbers were determined using tenfold dilutions made with sterile 3% NaCl and the drop-count plate method of Miles and Misra (1938) using Trypticase soy agar made up in 3% NaCl. All plate counts were made in quintuplicate with an incubation temperature of 25°C.

Chedabucto Bay sediment samples were collected aseptically with sterile polyvinyl chloride (PVC) tubes (2.5 cm ID × 25 cm length) beveled at the contact end to facilitate entry. Both ends of the tubes were closed with sterile rubber stoppers which were removed only for sample collection and replaced immediately. The tubes were driven by hand directly into shallow-water sediments or for deep-water samples into sediment materials brought to the surface using a van Veen grab sampler. Each tube sample was frozen with liquid nitrogen immediately and subsequently stored at normal freezer temperatures (−15 to −20°C) for later analysis.

Each sample was analyzed microbiologically in triplicate using the modified minimum dilution technique for determining the number of hydrocarbon-utilizing bacteria as a percentage of the total population as described by Mulkins-Phillips and Stewart (1974a).

The samples were thawed rapidly as previously described and 0.5 g of sediment was added to 4.5 mL of each type of medium and through tenfold dilutions carried to a final value of $10^{-6}$ and incubated at room temperature for a period of 4 wk. Parallel checks and confirmation of the results from the modified minimum dilution technique were provided by use of the hydrocarbon utilizer method (HCU) described by Mulkins-Phillips and Stewart (1974a).

Bacteria from the hydrocarbon-containing medium giving positive results were isolated successively on Trypticase soy agar plates containing 3% NaCl and basal medium agar plates (Mulkins-Phillips and Stewart 1974a) exposed to hydrocarbon vapors. Identification of the bacteria was carried out by methods described by Shewan et al. (1960) with reference to *Bergey's Manual* (Buchanan and Gibbons 1974).

## Results

Freezing sediment samples with liquid nitrogen followed by storage at temperatures ranging between −15 and −20°C appears to be a valid method for preserving large numbers of samples for later microbiological analysis (Fig. 1 and 2). Although relatively wide fluctuations are apparent, the results for the various storage temperatures and times compare well with the numbers reported for the unfrozen control samples.

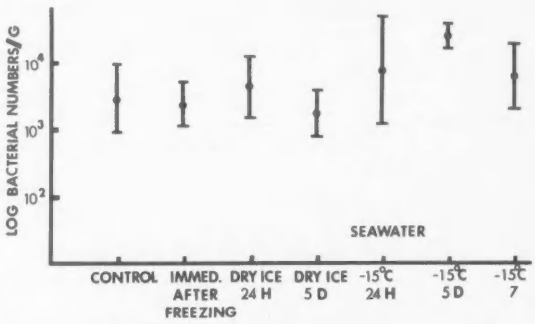


FIG. 1. Microbiological analysis of seawater collected aseptically at Point Pleasant Park, Halifax, September 14, 1977. The seawater was divided and one set of five replicates was analyzed immediately for total bacterial numbers and the remainder was frozen with liquid nitrogen and analyzed after times, storage modes, and storage temperatures indicated. Incubation temperature was 25°C; each point ± SD represents the mean for 25 measurements.

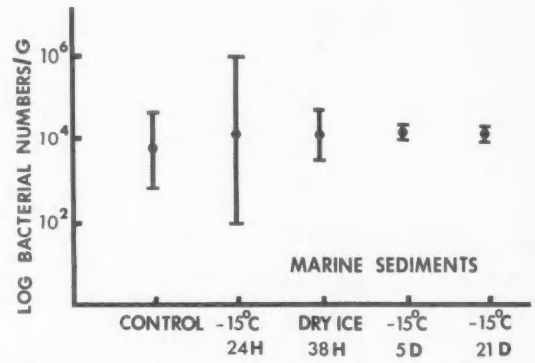


FIG. 2. Microbiological analysis of marine sediments collected aseptically at Point Pleasant Park, Halifax, December 15, 1976. The sediments were divided and treated as in Fig. 1.

The microbiological analysis of the Chedabucto Bay sediment samples determined by the modified minimum dilution method and confirmed by the HCU method are illustrated in Fig. 3. The total microbial numbers were similar to and consistent with the numbers for Chedabucto Bay and selected clean areas reported previously by Mulkins-Phillips and Stewart (1974a). In the current survey only two stations gave HCU populations that could be considered higher than normal. One, a shore sample, had a value of 40.5% hydrocarbon utilizers and the other, a deep-water sediment sample,

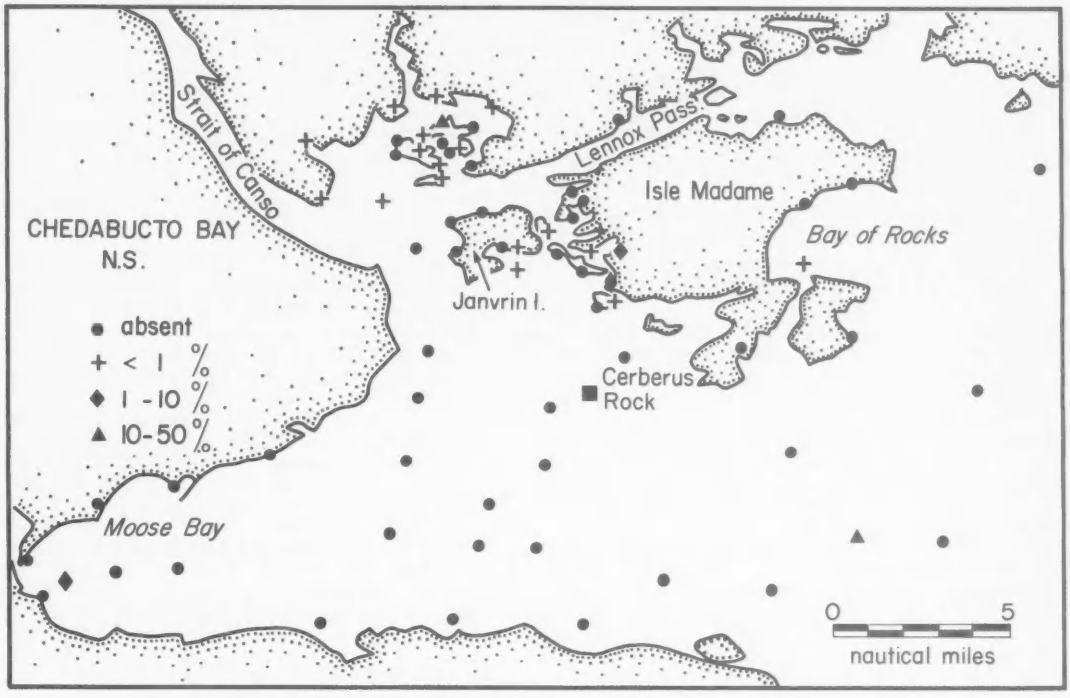


FIG. 3. Results of survey for hydrocarbon-utilizing microorganisms in sediments collected in July 1976 in Chedabucto Bay, N.S. Values are reported as HCU percentages of the total bacterial populations.

had a value of 12%. Of the remaining samples three had values for HCU microorganisms as a percentage of the total population of 2.0, 2.4, and 5.5% respectively; the rest had either none or <1%.

Identification of the bacteria isolated from 15 of the Chedabucto Bay sediment samples were classified to the generic level. The genera found were comparable to those found in previous studies. The list included *Pseudomonas* (group II), *Achromobacter*, *Flavobacter*, *Micrococci*, *Vibrio*, *Brevibacter*, several *Acinetobacter*-like microorganisms, and a number of fungi.

## Discussion

Past surveys and extensive experimental work have established that the indigenous population of hydrocarbon-utilizing microorganisms plays a major role in cleaning up spilled oil and can serve as a useful index of oil pollution. When oil is introduced to an environment the proportion of HCU microorganisms in the population increases; as the metabolically usable fractions disappear the HCU percentages decrease until they reach the usual background levels.

Application of this standard to Chedabucto Bay would indicate that in the sediments most of the hydrocarbons degradable by microorganisms had largely disappeared some 18 mo after the spill. Sediment samples taken from Chedabucto Bay in July and November of 1970 gave HCU percentage values ranging up to 15% of the population (Mulkins-Phillips and Stewart 1974a). When those areas were sampled again in the summers of 1971 and 1972, the numbers had fallen to around the 1% level, a value obtainable routinely in "clean" areas. Data obtained in the more extensive 1976 survey (Fig. 3) show that the numbers of HCU microorganisms were, with two exceptions, consistent with the 1971 and 1972 results reported by Mulkins-Phillips and Stewart (1974a). The conclusion that Chedabucto Bay is now relatively oil-free is confirmed by the results of the chemical analyses performed on the same sediment samples (Keizer et al. 1978). The abnormally high HCU populations of two of the stations could result from remnants of the 1970 Bunker C fuel oil spill or quite possibly from more recent and very local minor spills.

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# Prudhoe Crude Oil in Arctic Marine Ice, Water, and Sediment Ecosystems: Degradation and Interactions with Microbial and Benthic Communities[1]

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ATLAS, R. M., A. HOROWITZ, AND M. BUSDOSH. 1978. Prudhoe crude oil in arctic marine ice, water, and sediment ecosystems: degradation and interactions with microbial and benthic communities. J. Fish. Res. Board Can. 35: 585–590.

A variety of in situ models were used to simulate oil spills in different arctic ecosystems. Numbers of oil-degrading microorganisms were found to increase after oil contamination. Oil contamination of sediment resulted in mortality of indigenous invertebrates. Recolonization of oil-contaminated sediments began shortly after oil contamination but benthic communities were significantly different in oil-contaminated sediment compared with the control, 2 mo after oil contamination. Petroleum hydrocarbons were degraded slowly. Ice greatly restricted losses of light hydrocarbons. Following initial abiotic losses, biodegradation of oil was limited and did not significantly alter the relative percentages of hydrocarbons in the residual oil. We concluded that petroleum hydrocarbons will remain in arctic ecosystems for prolonged periods after oil contamination.

*Key words:* oil, petroleum, contamination, arctic, microorganisms, benthos

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Les auteurs ont utilisé divers modèles in situ simulant des déversements de pétrole dans des écosystèmes arctiques différents. On constate que le nombre de micro-organismes capables de dégrader le pétrole augmente après contamination par le pétrole. La contamination du sédiment par le pétrole entraîne des mortalités chez les invertébrés indigènes. Une nouvelle colonisation des sédiments contaminés commence peu après le déversement de pétrole, mais les communautés benthiques sont nettement différentes dans le sédiment contaminé par le pétrole comparativement au témoin, 2 mo après contamination. La dégradation des hydrocarbures du pétrole est lente. La glace restreint considérablement les pertes d'hydrocarbures légers. Après des pertes abiotiques initiales, la biodégradation du pétrole est limitée et n'altère pas sensiblement les pourcentages relatifs d'hydrocarbures dans le pétrole résiduaire. Nous en conclusons que les hydrocarbures de pétrole demeureront dans les écosystèmes arctique longtemps après contamination.

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To date there have been no major oil-contamination incidents in arctic marine ecosystems, but development of Canadian and United States offshore oil reserves in the Beaufort Sea threatens to change this situation. Initial offshore development will be in nearshore areas of <30 m deep. Offshore well and pipeline structures will have to withstand extreme forces of ice, and ship transport of oil will be extremely hazardous. Environmental assessment studies point out the inevitability of accidental contamination of arctic ecosystems with petroleum hydrocarbons (Weller 1977).

During much of the year the Arctic Ocean is covered by several metres of ice, and oil contamination from oil-well blowouts may contaminate over-ice, open-water, or under-ice ecosystems, depending on time of year and location of such an occurrence. Failures of buried pipelines would initially contaminate benthic ecosystems. Oil initially contaminating one ecosystem may migrate into other areas. Similarly oil spilled in terrestrial areas may be transported into marine ecosystems.

Anticipating the inevitable contamination of arctic marine ecosystems, we have established miniature experimental oil spills to simulate a variety of contamination situations. These model experiments have been conducted in situ to simulate as closely as possible conditions of real contamination incidents. The model oil spillages have been used to study degradative changes of petroleum hydrocarbons spilled over ice, under ice, on water, and in sediment. Microorganisms capable of degrading petroleum hydrocarbons were enumerated in these studies. We also examined the recolonization of

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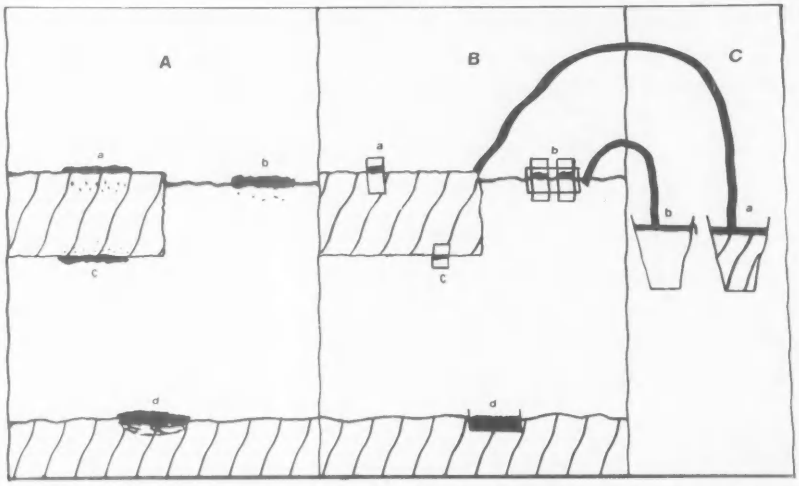


FIG. 1. Potential arctic oil spills: sites and models. A, Potential sites for oil contamination; B, in situ models for oil-spill assessments; C, models incubated in situ for oil-spill studies. a, Over ice; b, on water; c, under ice; d, sediment.

oil-contaminated sediment by benthic invertebrates. These studies should establish a predictive capability for the fate of petroleum hydrocarbons in arctic marine ecosystems and the effects of petroleum contamination on some indigenous organisms.

## Materials and Methods

### EXPOSURE OF ARCTIC ECOSYSTEMS TO CRUDE OIL

Figure 1 is a schematic of potential oil spill conditions in the arctic. Part A shows potential actual spill situations (a = over ice, b = on water, c = under ice, d = in sediment). Parts B and C show experimental in situ systems used to simulate the potential spill situations (a, b, c, d, defined as above).

The experimental sites were located near Point Barrow, Alaska. For modeling oil over ice spills, two systems were used. In the first (Fig. 1 Ba) ice cores, 8 cm diam × 30 cm long, were removed from sea ice, placed in Plexiglas cylinders that were stoppered on the bottom and replaced in situ in the ice. One millilitre Prudhoe crude oil was then added to each cylinder. Replicate cylinders were recovered semiweekly during a 4-wk period, two per sampling time, beginning in early May. In the second system (Fig. 1 Ca) 150 mL Prudhoe Bay crude oil was spilled over 1100-cm² surface area seawater (15 L volume) in plastic-lined chambers. The oil was applied in October and the seawater immediately allowed to freeze with most of the oil on the surface and the remainder in the ice matrix. The oil and ice were incubated in situ (i.e. exposed on the shore of the Arctic Ocean) from October to May. For simulating oil on water spills (Fig. 1 Bb), Plexiglas tubes, 5 cm diam × 25 cm long, were floated on open water during July and August. One millilitre Prudhoe Bay crude oil was added to each cylinder. The bottoms of replicate cylinders were stoppered and duplicate cylinders recovered weekly for 2 mo. Also, for simulating oil on water spills, an open flow-through system (Fig. 1 Cb) was used (Horowitz and Atlas 1977). In the flow-through system, 150 mL Prudhoe Bay crude oil was applied to 1100-cm² surface area. The flow-through system was incubated in situ from June to September. For simulating under-ice oil spillages, stainless steel cylinders, 10 cm diam × 20 cm long, were implanted into the bottom side of the ice (Fig. 1 Bc). One millilitre Prudhoe Bay crude oil was injected into the cylinders by SCUBA divers. The cylinders were held in place in situ with Styrofoam flotation collars. Under-ice experiments were conducted during May. For simulating oil in sediment, 100, 25 × 25 × 7 cm trays were filled to 2 cm deep with freshly collected sediment from Elson Lagoon (Fig. 1 Bd). Benthic animals were physically removed from trays for recolonization studies. Trays with no animals removed were used for mortality studies. Two hundred millilitres Prudhoe Bay crude oil was added to the sediment and the trays replaced in situ at approximately 5 m deep. A Plexiglas lid was held over the trays during transport to the bottom by SCUBA divers. Oil-in-sediment experiments were begun in May and were followed for 60 d.

### ANALYSIS OF OIL DEGRADATION

Oil was recovered after in situ exposure by solvent extraction with diethyl ether. Oil exposed on water or under ice was recovered with a separatory funnel. Ice was allowed to melt before extraction. For recovery of oil from sediment, sediment was mixed with a glass stirring rod in an attempt to evenly distribute the residual oil. Five sediment samples, ca 150 g each, were removed from each tray. Solvent was repeatedly mixed with sediment samples, and decanted. Solvent extracts were concentrated under an air-stream, dried with sodium sulfate, and brought to constant volume.

Recovered oils were analyzed by gas–liquid chromatography with a Hewlett Packard model 5830A reporting gas chromatograph with 2-m columns packed with 3% OV1 on Chromosorb W 80–100 mesh. Temperature was programmed at 80°C isothermal for 8 min, increased 8°C/min to 250°C, and held isothermal at 250°C for 20 min.

Relative percentages of oil components were automatically calculated. Some extracted oils were further analyzed by evaporating the solvent and weighing the residual oil. Some residual oils were also analyzed by column-chromatographic and mass-spectral analyses of hydrocarbon classes.

### Enumeration of Microorganisms

Microbial populations were enumerated before and after exposure to petroleum hydrocarbons. Estimation of hydrocarbon-utilizing microorganisms was made using oil agar media with 1% Prudhoe Bay crude oil (Atlas and Bartha 1973). Viable heterotrophic microorganisms were enumerated with marine agar 2216. Incubation at 5°C for 1 mo was used for enumeration of these populations. Ratios of oil-utilizing microorganisms to viable heterotrophs were calculated.

Only some representative numbers of oil-degrading microorganisms and rates of oil utilizers to total heterotrophs before and after exposure to Prudhoe Bay crude oil are included in this paper. Over-ice, open-water, and under-ice populations were enumerated from the in situ contained-cylinder exposures. For over-ice populations a 2.5-cm depth of ice core was removed, allowed to melt at 5°C, and used for enumerations. For under ice a 2.5-cm depth of ice core and a 2.5-cm depth of underlying water were removed, the ice allowed to melt at 5°C, and enumerations performed on the pooled sample. Over-water samples were obtained from just under the oil layer. For sediment the top 1 cm from the sediment trays was used. For all enumerations two determinations each of countable dilutions or filtrations on duplicate cores were averaged.

### Benthic Community

Recolonization of oil-contaminated sediment by benthic invertebrates was studied by recovering animals from the exposed oil-treated and control trays. A sieve, 4 mm hole diam, was used for recovering animals. Recovered animals were identified and quantitated. Four replicate trays were used for each determination.

## Results and Discussion

### Microbial Oil-Degrading Populations

Microorganisms capable of degrading hydrocarbons were present in all the arctic marine ecosystems we examined (Table 1). In non-oil-contaminated ecosystems, the oil-degrading microbial population constituted only a small percentage of the viable heterotrophic microorganisms. Following exposure to oil for 30 d, the estimated percentage of oil-utilizing microorganisms rose greatly in over-water experiments, but only minimally in over- and under-ice experiments. Concentrations of oil-utilizing microorganisms were lower in over- and under-ice ecosystems than in open-water or sediment ecosystems. Detailed population studies on the flow-through oil on-water and ice exposures have been reported elsewhere (Horowitz and Atlas 1977, 1978) and show similar changes to those included in Table 1. Unfortunately, measurements of oil-utilizing microorganisms were not made for sediment after oil contamination. Our prediction, based on work in nearby saline lake sediment, is that there was a significant rise in the percentage of heterotrophs capable of utilizing petroleum hydrocarbons in the oil-contaminated sediment. In general, contamination of an arctic marine ecosystem with petroleum hydrocarbons results in an increased number of viable heterotrophic and oil-utilizing microorganisms and an increased percentage of oil-utilizing microorganisms in the heterotrophic microbial community. The petroleum hydrocarbons appear to remove a limitation of available organic carbon for the growth of microorganisms in these ecosystems. The response is most limited in-, on-, or under-ice ecosystems where there may be other nutrient or temperature limitations for microbial productivity.

TABLE 1. Microbial populations before and 30 d after oil contamination.

| | Oil-Utilizing Microorganisms (no./mL or no./g) | | | |
|---|---|---|---|---|
| | Before oil contamination | | After oil contamination | |
| Ecosystem | Estimated no. | Estimated percent of heterotrophs | Estimated no. | Estimated percent of heterotrophs |
| Over ice | $1\times10^{-1}$ | 0.01 | $3\times10^{-1}$ | 0.03 |
| Open water | $5\times10^{1}$ | 0.5 | $7\times10^{3}$ | 50.0 |
| Under ice | $1\times10^{0}$ | 0.1 | $5\times10^{0}$ | 0.5 |
| Sediment | $3\times10^{3}$ | 0.1 | — | — |

### Effect of Oil on Benthic Community

We found that application of Prudhoe crude oil resulted in rapid death or movement from oil-contaminated sediment of the indigenous benthic invertebrates. To simulate this observed situation, we studied the recolonization of oil-contaminated and unoiled sediment after physical removal of the invertebrate organisms. Within 1 wk, attempts at recolonization by invertebrates were observed. Several organisms, e.g. a burrowing anemone (unidentified sp.) and a bivalve (*Liocyma* sp.), were found in control and oil-contaminated trays. These organisms died, however, within 2 wk in the oil-contaminated sediment, but remained alive for over 1 mo in the unoiled sediment. After 60 d, neither the bivalve nor the anemone was found in control or oil-contaminated trays. We are unable to explain the disappearance of these organisms from the control trays at the 60-d sampling time. The 60-d samples were collected shortly after several severe storms, which may have disturbed the benthic community.

The 60-d samples did show some interesting differences in the recolonization of oiled and control sediment (Table 2). Amphipod species were found much less frequently in oil-contaminated than control sediments. Contact with Prudhoe Bay crude oil has previously been found to be toxic to several different amphipods from this area (Busdosh and Atlas 1977). Isopods were found as frequently in oil-contaminated sediment as they were in control sediment. Percy and Mullin (1975) reported that the isopod found in our study shows little preference for oil-tainted or untainted sediments and is ex-

TABLE 2. Benthic recolonization 60 d after oil contamination.

| Organism | Mean no./m² | |
|---|---|---|
| | Control | Oil |
| Amphipoda | | |
| *Pontoporeia femorata* | 16 | 6 |
| *Aceroides latipes* | 16 | 0 |
| *Melita formosa* | 14 | 1 |
| *Gammaracanthus loricatus* | 2 | 0 |
| *Monoculodes* sp. | 20 | 4 |
| Isopoda | | |
| *Saduria entomon* | 4 | 5 |
| Polychaeta | | |
| *Acanthostephieia behrengiensis* | 4 | 0 |
| *Pectinaria hyperborea* | 0 | 5 |
| *Nephythys longosetosa* | 0 | 4 |
| *Spio* sp. | 0 | 4 |

tremely resistant to crude oil dispersions. With respect to polychaetes, some species were found in oil-contaminated, but not control, sediments and one species found in controls was absent from oil-contaminated sediment. Some polychaete species may be attracted to oil, while oil is toxic to other species. Carr and Reish (1977) found the degree of susceptibility of polychaetes to petroleum to be species dependent. Overall, oil-contaminated sediment is recolonized by invertebrates within 2 mo of contamination. The benthic community composition, however, is markedly different in oil-contaminated than control sediment 60 d after contamination. Thus, while relatively rapid "recovery" is likely after oil contamination of arctic benthic ecosystems, relatively long-lasting changes in the benthos can be predicted.

## OIL DEGRADATION

Prudhoe crude oil was found to be degraded only relatively slowly in most of the ecosystems we examined. When spilled on water during summer, there was a 22% loss in 1 mo, but only a 25% loss in 2 mo on a weight basis. During the 1st mo of exposure, oil spilled on water lost all light hydrocarbons, below a retention time of 17 min (Fig. 2). The loss of light hydrocarbons was probably almost entirely due to abiotic evaporative weathering. During the following month, only relatively minor changes occurred in the composition of the residual oil. Compositional changes during the 2nd mo of exposure probably are due to slow, limited biodegradation. Oil spilled over ice showed only a 5% weight loss during 9 mo of exposure over the winter. The gas–liquid chromatographic analysis showed a loss of light hydrocarbons below retention times of 8 min (Fig. 3). The relative concentrations of hydrocarbons in the residual oil indicated that no biodegradation of oil over ice had occurred during winter. The comparison

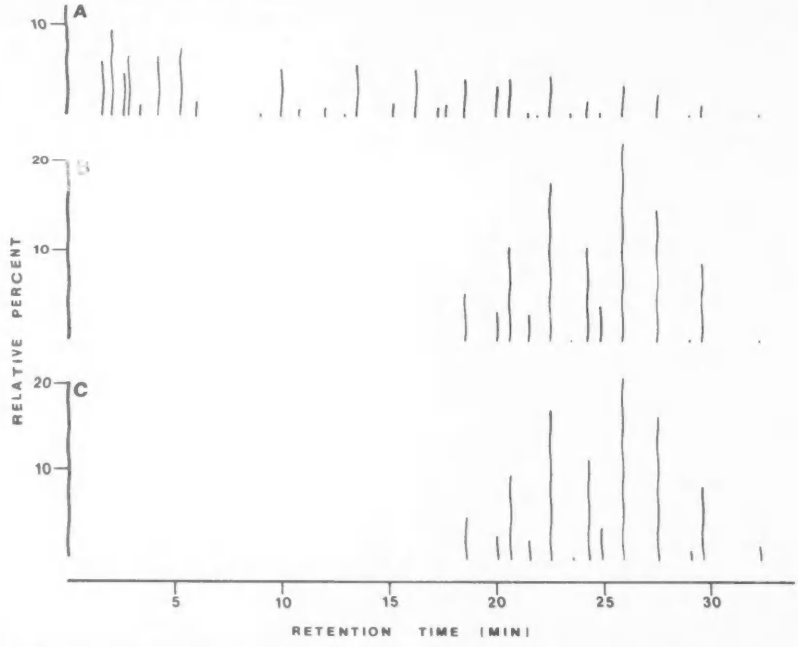


FIG. 2. Gas–liquid chromatographic analysis of oil spilled on water. A, Prudhoe Bay crude oil; B, residual oil after 1-mo exposure; C, residual oil after 2-mo exposure.

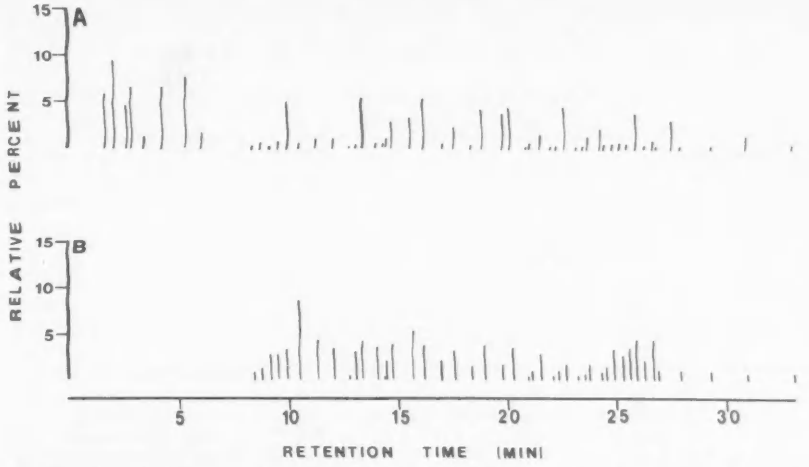


FIG. 3. Gas–liquid chromatographic analysis of oil spilled over ice. A, Prudhoe Bay crude oil; B, residual oil after 9-mo exposure.

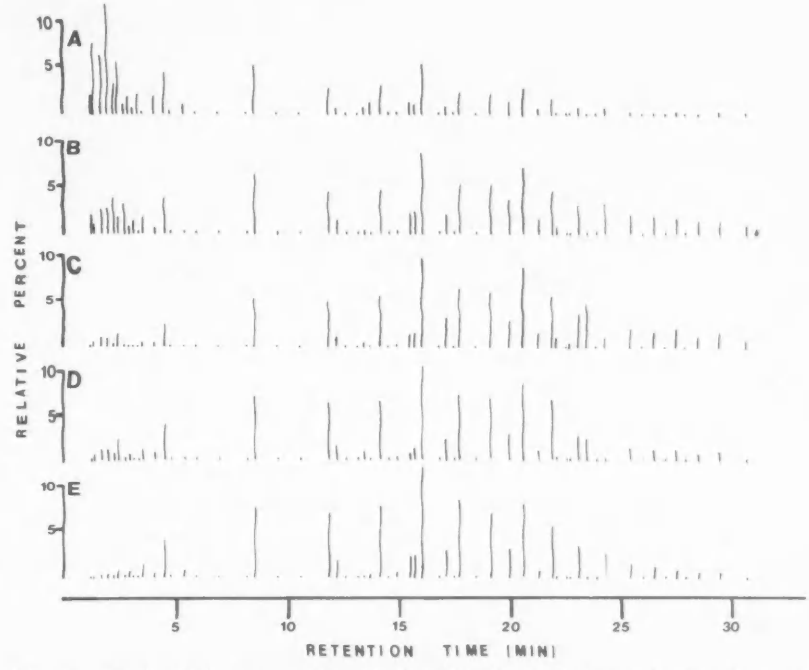


FIG. 4. Gas–liquid chromatographic analysis of oil spilled under ice. A, Prudhoe Bay crude oil; B, residual oil after 1-d exposure; C, residual oil after 5-d exposure; D, residual oil after 14-d exposure; E, residual oil after 21-d exposure.

of Fig. 2 and 3 clearly shows that less degradative changes occur during winter for oil over ice than during summer for oil on water. When spilled under ice, there were only slow compositional changes (Fig. 4). Only 75% of the weight of the oil was recoverable 1 d after spillage of oil under ice. We believe that the loss was physical, probably occurring during handling, transport, and recovery of the ice cores. The residual oil from the under-ice experiments showed a gradual disappearance of light hydrocarbons. However, even after 3-wk exposure, significant quantities of light hydrocarbons remained in the oil. The ice cover probably

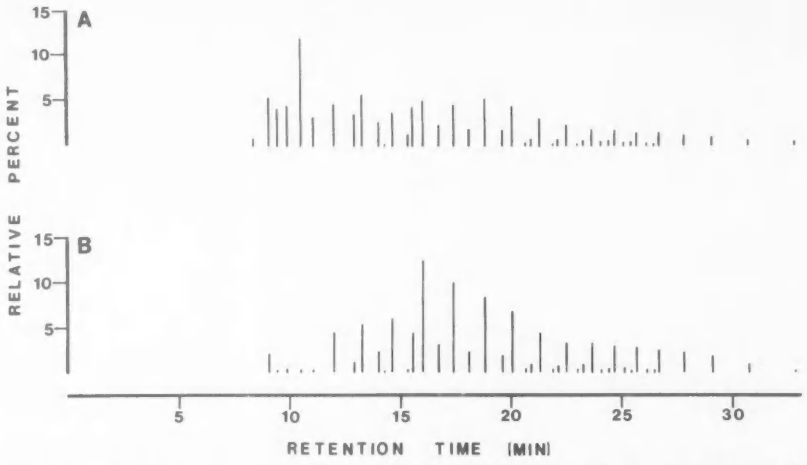


FIG. 5. Gas–liquid chromatographic analysis of oil spilled in sediment. A, Residual oil after 2 d; B, residual oil after 60 d.

limits evaporative losses, and dissolution probably accounts for the loss of light hydrocarbons. If the toxic fraction of crude oil resides in this light fraction, oil spilled under ice will retain toxic properties for prolonged periods. In sediment there was a 45% weight loss after 60 d compared with oil remaining after 2 d. Technical problems prevented recovery of oil from contaminated sediment at 0 time. Even after 2 d, light hydrocarbons were not found in the oil recovered from the sediment (Fig. 5). During 60 d of exposure, there were major changes in the composition of oil in sediment. These changes are probably due to biodegradation. However, petroleum hydrocarbons will likely remain in contaminated sediment for a long time. When exposed for a similar 2-mo period, greater concentrations of hydrocarbons of low molecular weight remained in oil exposed in sediment than in oil exposed on water. These sediment studies are ongoing to examine the long-term fate of oil in arctic marine sediments.

Generally, rates of degradation of petroleum hydrocarbons in arctic ecosystems appear to be very slow. Following initial abiotic weathering, biodegradation occurs slowly. When exposed on water, biodegradation reduces absolute amounts of petroleum hydrocarbons, but does not appear to alter the relative percentages of oil components. This has been shown with column chromatographic and mass spectral analyses (Horowitz and Atlas 1977). It is not known if biodegradation of oil in sediment will also show a pattern of similar relative degradation rates for different classes of hydrocarbons. However, it can be generally stated that organisms in oil-contaminated ecosystems will have to tolerate the presence of undegraded petroleum hydrocarbons for extended periods of time, migrate from the area, or perish.

## Acknowledgments

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# Importance of Sediment Working by the Deposit-Feeding Polychaete *Arenicola marina* on the Weathering Rate of Sediment-Bound Oil[1,2]

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GORDON, D. C. JR., J. DALE, AND P. D. KEIZER. 1978. Importance of sediment working by the deposit-feeding polychaete *Arenicola marina* on the weathering rate of sediment-bound oil. J. Fish. Res. Board Can. 35: 591–603.

The interactions between the polychaete *Arenicola marina*, a common deposit feeder in sandy intertidal areas, and sediment contaminated with the fresh API (American Petroleum Institute) reference oils and weathered Bunker C oil remaining from the 1970 *Arrow* spill were investigated in laboratory experiments. Worms can tolerate low concentrations of sediment-bound oil, although the sediment working rate is reduced depending upon oil type, concentration, and degree of weathering. Hydrocarbon concentrations, measured gravimetrically and by gas chromatography and fluorescence spectroscopy, were substantially lower (17–72%) in worm casts than in initial sediment. This loss can be accounted for by microbial degradation, which is stimulated by the worms' activity, uptake of hydrocarbons into worms, and perhaps dissolution. A large population (10–25/m²) of *Arenicola* is present at Black Duck Cove, N.S., living in sediment contaminated with weathered Bunker C oil remaining from the *Arrow* spill. These worms, which have spent their entire lives in oil-polluted sediment, do not have markedly elevated hydrocarbon concentrations and behaved no differently in experiments than worms collected from a clean environment. Calculations suggest that they are capable of removing the oil present in a square metre of sediment in 2–4 yr. After any oil spill, when concentrations reach tolerable levels, activities of deposit-feeding animals such as *Arenicola* can apparently accelerate the weathering rate of sediment-bound oil.

*Key words:* sediment, *Arenicola marina*, polychaete, petroleum, weathering rate, pollution, hydrocarbon

GORDON, D. C. JR., J. DALE, AND P. D. KEIZER. 1978. Importance of sediment working by the deposit-feeding polychaete *Arenicola marina* on the weathering rate of sediment-bound oil. J. Fish. Res. Board Can. 35: 591–603.

Nous avons étudié au moyen d'expériences en laboratoire les interactions entre le polychète *Arenicola marina*, un organisme se nourrisant de dépôts et commun dans les régions intertidales sablonneuses, et les sédiments contaminés par les pétroles de référence frais de l'API et par le fuel de soute C altéré à l'air, reliquat du deversement du pétrolier *Arrow* en 1970. Les vers peuvent tolérer de faibles concentrations de pétrole lié au sédiment, bien que le taux de remuement du sediment soit réduit selon le type, la concentration et le degré d'altération du pétrole. Les teneurs en hydrocarbures, mesurées par gravimétrie et par chromatographie en phase gazeuse et spectroscopie de fluorescence, sont substantiellement plus faibles (17–72%) dans les déjections des vers que dans le sédiment initial. Cette perte peut s'expliquer par dégradation microbienne, stimulée par l'activité des vers, par l'assimilation des hydrocarbures dans les vers et peut-être par la dissolution. Il y a une abondante population (10–25/m²) d'*Arenicola* à Black Duck Cove, N.-E., qui vit dans un sédiment contaminé par du fuel de soute C altéré à l'air restant du deversement de l'*Arrow*. Ces vers qui ont passé leur vie entière dans un sédiment contaminé par le pétrole n'ont pas de teneurs notablement élevées en hydrocarbures et, lors d'expériences, n'ont pas de comportement différent de celui des vers prélevés dans un environnement propre. Des calculs suggèrent qu'ils sont capables d'enlever dans 2–4 a le pétrole présent dans un mètre carré de sédiment. Après un déversement de pétrole, quand les concentrations atteignent des niveaux tolérables, il semble que les activités d'animaux se nourrissant de dépôts, tel *Arenicola*, peuvent accélérer le taux d'altération du pétrole lié au sédiment.

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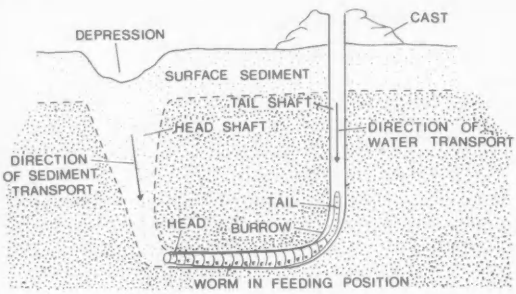


FIG. 1. Schematic diagram of *Arenicola marina* burrow (not to scale). The lightly stippled area denotes oxidized sediment, the heavily stippled area reduced sediment.

ONCE incorporated into sediments of low energy, cold-water environments, spilled oil can persist for many years. For example, substantial amounts of Bunker C fuel oil from the 1970 *Arrow* spill can still be found in Chedabucto Bay, N.S. (Keizer et al. 1978). During the very slow weathering process, sediment-bound oil could be in continuous contact with benthic organisms. The benthos, especially in intertidal areas, is very likely to demonstrate long-term effects from oil spills. Of particular interest is the response of deposit-feeding organisms which turn over large quantities of sediment during their brief life spans.

In 1975 we began a study of the interactions between the deposit-feeding polychaete *Arenicola marina* and sediment-bound oil. Our intention was twofold. First, how does the presence of different concentrations and types of oil in sediment affect the general condition and activity of worms? And second, how does the sediment-working activity of worms, if not prevented by the presence of oil, alter the hydrocarbon chemistry of sediment?

The preliminary experiments, which employed only fresh No. 2 fuel oil from the API reference collection, have been discussed by Prouse and Gordon (1976). In summary, we found that sediment oil concentrations on the order of 250 $\mu$g/g completely stopped sediment-working activity in some worms and substantially reduced the rate of activity in others. Analysis of initial sediment and casts by fluorescence spectroscopy indicated that substantial amounts of aromatic hydrocarbons were being removed from the sediment during the working process. This observation suggested that sediment working by deposit-feeding organisms such as *Arenicola* could be an important factor affecting the weathering rate of sediment-bound oil.

This paper reports the results of more detailed laboratory experiments employing all four of the API reference oils (No. 2 fuel, Venezuelan Bunker C, South Louisiana crude, and Kuwait crude) plus weathered Bunker C oil remaining from the 1970 *Arrow* spill. In addition, field observations were made at two sites, one unpolluted and the other polluted with *Arrow* Bunker C. At the latter site, worms have been exposed to oil for at least their entire life span and perhaps several generations. More exacting chemical methods have been employed to examine changes in sediment-hydrocarbon chemistry, resulting from working activity, and the uptake of hydrocarbons by worms.

## Natural History of *Arenicola marina*

*Arenicola marina*, a large polychaete commonly found in sandy intertidal areas on both sides of the North Atlantic, is one of 24 species and subspecies of lugworms, which are widely distributed around the world (Wells 1964). Their burrowing and feeding habits are similar and well documented (Wells 1945; Jacobsen 1967; Hylleberg 1975; Cadée 1976). In brief, lugworms occupy L-shaped burrows which generally range between 20 and 40 cm deep (Fig. 1). As the worm ingests sediment at the bottom of the headshaft (2 mm is about the largest particle that can be ingested), surface sediment collapses from above, which causes a funnel-like depression to form at the sediment surface. This depression can be observed in the field and its exact form is dependent upon the physical properties of the sediment. Despite the fact that lugworms are feeding at a depth of 20–40 cm, most of the sediment they ingest originates from the surface. To defecate, the worm moves backward in the tailshaft, until its tail reaches the sediment surface, and discharges the ingested sediment around the burrow opening. All sediment discharged passes through the worm's gut; no pseudofeces are produced. Individual worms can work in excess of 50 g (wet) of sediment daily or about 10 times their own wet weight. The sediment-working rate can be highly variable with time, and rest periods as long as several days can occur. When worms are active, casts are generally produced about once per hour. Nutrition is thought to be derived from microorganisms, nematodes, and detritus contained in the sediment. While transporting sediment in one direction through its burrow, the worm also pumps seawater in the other (Fig. 1). This water provides oxygen for respiration and ensures that the sediment lining the burrow and filling the headshaft remains oxidized. Although surrounded on most sides by reduced sediment, the worm lives in an oxidized environment. Population densities in some localities can exceed 100 worms/m$^2$ (Cadée 1976).

## Materials and Methods

### FIELD SAMPLING

Worms and sediment were collected between May and August 1977 at low tide from intertidal flats at Petpeswick Inlet, an unpolluted estuary about 45 km east of Halifax, and from a tidal lagoon in Black Duck Cove, near Canso, N.S. The latter site was heavily oiled with Bunker C from the *Arrow* in the spring of 1970. Worms were dug by shovel and placed immediately in separate 400-mL beakers containing seawater. Sediment was shoveled into plastic buckets and covered with seawater for transit back to the laboratory.

TABLE 1. Summary of experimental conditions. N2F = No. 2 fuel, VBC = Venezuelan Bunker C, SLC = South Louisiana crude, and KC = Kuwait crude; all from the API reference collection. ABC = *Arrow* Bunker C. Oil concentrations determined gravimetrically.

| Expt | Dates | Temp range (°C) | Oil(s) used | Sediment mean oil concn (μg/g) | Origin of sediment and worms | Sediment stratification |
|---|---|---|---|---|---|---|
| 1 | May 2–6 | 5.0–5.6 | N2F, VBC, SLC, KC | 37 | Petpeswick | None |
| 2 | May 8–12 | 3.7–5.5 | N2F, VBC, SLC, KC | 87 | Petpeswick | None |
| 3 | May 15–19 | 6.0–7.7 | N2F, VBC, SLC, KC | 153 | Petpeswick | None |
| 4 | June 9–13 | 10.0–12.0 | N2F, VBC, SLC, KC | 182 | Petpeswick | None |
| 5 | June 16–20 | 8.0–9.0 | N2F, VBC, SLC, KC | 275 | Petpeswick | None |
| 6 | June 24–28 | 8.5–9.0 | VBC | 79–2086 | Petpeswick | Oiled sediment upper 8 cm |
| 7 | July 1–15 | 12.0–16.0 | ABC | 190–800 | Black Duck Cove and Petpeswick | None |
| 8 | July 30–Aug. 12 | 10.0–19.0 | ABC | 95–260 | Black Duck Cove and Petpeswick | Oiled sediment upper 5 cm |
| 9 | Aug. 16–24 | 13.5–23.5<br>12.0–15.0<br>0.0–5.0 | VBC | 35 | Black Duck Cove (worms) and Petpeswick (sediment) | None |

## EXPERIMENTAL

Upon return to the laboratory (within 4 h), the beakers containing the worms were placed in a tank of running seawater. Sediment (8–16 L at a time) was placed in a large plastic tub (rinsed with methylene chloride between uses) and picked clean of macroinvertebrates, shells, and large pieces of plant material. Oil (except for control sediment) and seawater were added and the sediment, having a consistency similar to mortar, was mixed by hand for about 15 min. Sediment was then transferred to 2-L glass beakers (18 cm deep) which were filled to within 2 cm of the rim. Four replicate beakers of each sediment mixture were prepared for experiments using worms from one location, while eight replicates were prepared for experiments comparing worms from both sampling locations. Each set of replicates was placed in a separate 70-L tank containing running seawater (salinity 30‰; flow rate approximately 5 L/min), the level of which was 6–10 cm above the beakers.

The next day, after worms had completely voided their guts of sediment, each worm was rinsed free of sediment clinging to its surface, blotted dry on paper toweling, and weighed. After the most robust worms were selected, experiments were started by adding one worm to each sediment beaker. This procedure was altered only in Experiments 6 and 8, which included stratified oil concentrations. In these cases, oiled surface sediment was not added until the worms had burrowed into the clean sediment in the lower portion of the beakers.

Each beaker was removed from its tank daily (at approximately the same time) and the surface water was drawn off with a disposable plastic syringe, being careful not to disturb the casts. Casts were removed from the sediment surface with a stainless steel spatula and placed in a tared aluminum weighing dish (spatula and dish were rinsed with pentane if the casts were to be analyzed chemically). Casts were weighed wet (dry sediment weight being 70% wet weight).

Nine experiments were run (Table 1). At the end of each, after the necessary cast and sediment samples were collected, worms were recovered, put back into 400-mL beakers, and placed again in a running-seawater tank. Casts (after weighing) and sediment samples from each set of replicate beakers were combined and composite samples of 50 g each were taken for chemical analysis (in some instances, combined cast samples were <50 g, in which case the entire sample was analyzed). After 24 h when the worms had emptied their guts of sediment, they were reweighed. Samples not extracted immediately were frozen in pentane-rinsed glass containers.

## ANALYTICAL

Grain-size analysis of sediment and casts was conducted with wet-sieving techniques.

Sediment and cast samples were extracted with pentane in the presence of preextracted Super Q water (2.5 mL pentane:0.5 mL $H_2O$/g wet sediment) by agitating 15 min on a wristaction shaker. After overnight storage at 4°C, the emulsion was broken by centrifugation and the organic phase decanted off. After concentration to 1 mL with a Kuderna–Danish concentrator, pentane-extractable organic matter (PEOM) was determined by drying a 50-μL aliquot under nitrogen in a tared aluminum boat and weighing the residue on an electrobalance.

Worms (composite sample of two, generally 4–6 g wet each) were homogenized for 60 s with 50 mL methanol–benzene (40:60) with a Polytron homogenizer. After filtration through prerinsed Whatman No. 1 filter paper, the filtrate was washed with 30 mL acidic saturated NaCl (preextracted 3 times with pentane). The benzene layer was separated and the aqueous layer was extracted twice with pentane. After washing the combined pentane extract with acidic saturated NaCl, the organic phase was separated, concentrated to 25 mL, and PEOM determined with a 50–100-μL aliquot. Worm extracts were refluxed in boiling methanolic KOH for 2 h, after which the above extraction procedure was repeated. The nonsaponifiable PEOM was measured with another 50–100-μL aliquot.

Sediment and worm extracts were then analyzed by fluorescence spectroscopy (Gordon and Keizer 1974). Quantitative estimates were obtained by using the appropriate oil as a standard, and qualitative information was

TABLE 2. Averaged results of experiments designed to determine the recovery of oil from sediment by the analytical methods used in this project. Unoiled Petpeswick sediment was spiked with known amounts (30–300 μg/g) of the four API reference oils.

| Oil | % recovery | |
|---|---|---|
| | Gravimetric analysis | Fluorescence analysis |
| No. 2 fuel oil | 53 | 84 |
| Venezuelan Bunker C | 72 | 85 |
| South Louisiana crude | 68 | 96 |
| Kuwait crude | 63 | 92 |

obtained by running synchronous excitation of emission spectra (SEES).

Sediment extracts were chromatographed on 0.5-cm-diameter columns containing approximately 6 cm silica gel (baked overnight at 250°C and deactivated 5% with water) and 0.5 cm alumina (baked overnight at 400°C and deactivated 5% with water) which had been prerinsed with two bed volumes of benzene followed by pentane. The $f_1$ fraction (e.g. *n*-alkanes, cycloalkanes, isoprenoids, some alkenes) was eluted with one bed volume of pentane, the $f_2$ fraction (e.g. some alkenes, polycyclic aromatics) with one bed volume each of 10 and 20% benzene in pentane, and the $f_3$ fraction (e.g. high molecular weight polynuclearomatic hydrocarbons ((PAH's)) with one bed volume of benzene. Worm extracts were chromatographed on 0.9-cm-diameter columns 20 cm long. Silica gel and alumina (prepared as above) were used in a 1:1 ratio, the amount depending on the nonsaponifiable PEOM concentration of each extract (1 g total packing/10 mg PEOM). Columns were prerinsed with two bed volumes of methanol–benzene, one bed volume of benzene, and two bed volumes of pentane. Three fractions were collected as above. With both sediment and worm samples, the eluates of each fraction were concentrated to 1 mL with a Kuderna–Danish concentrator and the weight determined with a 50-μL aliquot.

Following further concentration of the $f_1$ and $f_2$ eluates to 100 μL, aliquots (generally 20 μL for sediments and 1 μL for worms) were transferred to aluminum capsules, concentrated to about 1 μL under nitrogen when necessary, and sealed. All samples were analyzed on the same glass capillary column (25 m, OV-101) fitted in a Perkin–Elmer 990 gas chromatograph equipped with a MS-41 injection assembly and a Model 3380A Hewlett Packard integrator (injector temperature 250°C, FID detector temperature 300°C; programmed from 100 to 260°C at 6°C/min). Compounds were tentatively identified by comparison of retention times with *n*-alkane standards.

All glassware was rinsed with solvent 3 times prior to use. All solvents were redistilled in glass. Frequent solvent blanks were prepared and analyzed and were always clean.

Recovery of petroleum hydrocarbons by the analytical procedure outlined above was checked by analyzing Petpeswick sediment spiked with known weights of each of the four API reference oils. The recovery of oil as measured gravimetrically (PEOM) was related to the volatility of the oil (Table 2). Except for Venezuelan Bunker C, of which only 75% is soluble in pentane, most of the loss occurred when aliquots were being evaporated under nitrogen in the aluminum boats just prior to weighing. The recovery of the aromatic hydrocarbons detected by fluorescence spectroscopy was quite good when expressed in whole oil units (Table 2). The recoveries of *n*-alkanes were low, averaging about 40%, and quite variable. Most of this loss was traced to insufficient rinsing of container walls when extracts were being concentrated to 100 μL in Reactivials just prior to encapsulation.

Except where noted, all concentrations presented are corrected with these recovery data and refer to wet-sediment weights. Unless otherwise stated, all total oil concentrations reported were determined gravimetrically and corrected for control concentrations (in the case of worms, the nonsaponifiable PEOM concentrations were used).

## Study Areas

The two intertidal flats sampled at Petpeswick Inlet consisted of a well-sorted, medium to fine sand (Table 3). PEOM and hydrocarbon concentrations were very low (Table 4). The *n*-alkanes displayed the strong odd-carbon preference typical of unpolluted coastal sediments, and no unresolved complex mixture (UCM) was detected (Fig. 2). The $f_2$ fraction was practically devoid of hydrocarbons (Fig. 3).

The sediment in the tidal lagoon at Black Duck Cove is a poorly sorted mixture of gravel, sand, silt, and clay (Table 3) (see Owens and Rashid [1976] for a thorough geological description of Black Duck Cove and a documentation of oiling conditions). A thick layer of stranded oil can still be found in 1977 in the upper intertidal zone around the lagoon. This oil is weathering slowly and moving into the central portion of the lagoon, presumably by the mechanism described by Vandermeulen and Gordon (1976). The presence of oil in the surface sediment of

TABLE 3. Grain-size analysis of composite samples of sediment and casts collected at Petpeswick Inlet and Black Duck Cove, N.S.

| Site | Sample | % weight | | | |
|---|---|---|---|---|---|
| | | Gravel (>1680 μm) | Coarse sand (500–1680 μm) | Medium and fine sand (63–500 μm) | Silt and clay (<63 μm) |
| Petpeswick Inlet | Sediment | 0 | 3 | 96 | 1 |
| | Cast | 0 | 1 | 98 | 1 |
| Black Duck Cove | Sediment | 24 | 40 | 34 | 2 |
| | Cast | 5 | 43 | 48 | 4 |

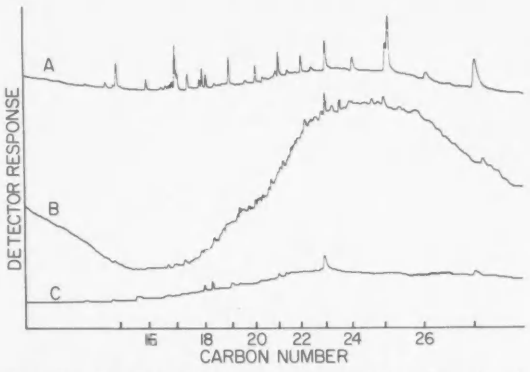


FIG. 2. Characteristic chromatograms of sediment collected from Petpeswick and Black Duck Cove, $f_1$ fraction. A, Petpeswick surface sediment, 20 μL, atten. (attenuation) 32; B, Black Duck Cove surface sediment, 1 μL, atten. 16; C, Black Duck Cove deep (15–20 cm) sediment, 10 μL, atten. 32.

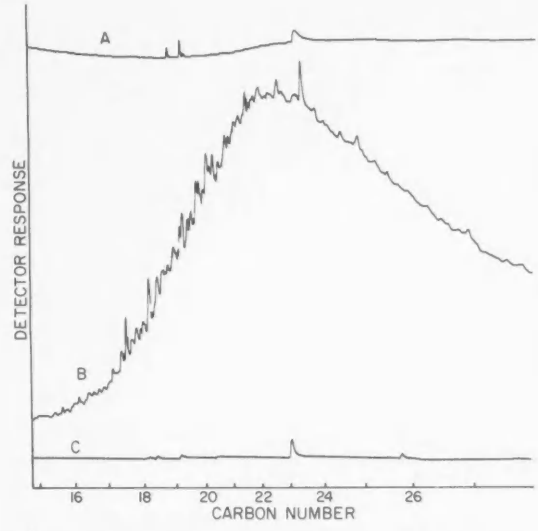


FIG. 3. Characteristic chromatograms of sediment collected from Petpeswick and Black Duck Cove, $f_2$ fraction. A, Petpeswick surface sediment, 5 μL, atten. 64; B, Black Duck Cove surface sediment, 1 μL, atten. 16; C, Black Duck Cove deep (15–20 cm) sediment, 20 μL, atten. 256.

the central portion is not visibly apparent at first glance. However, when digging samples, an iridescent sheen soon appears on the interstitial water. Oil concentrations in the surface sediments are quite variable and exceed 1 mg/g in some samples (Table 4). *n*-Alkane concentrations are very low, presumably because of the extensive weathering that has taken place during the 7 yr the oil has been stranded. Chromatograms of the $f_1$ and $f_2$ fractions contained few resolved compounds but large UCM's (Fig. 2 and 3). Only a trace of oil has penetrated the sediment

TABLE 4. Hydrocarbon chemistry of sediment from Petpeswick Inlet and Black Duck Cove, N.S. Range of concentrations observed between May and August 1977. "Oil" denotes estimates of oil concentration obtained by fluorescence spectroscopy; PEOM, pentane-extractable organic matter. Few, if any, hydrocarbons in Petpeswick sediment have a petroleum origin. Black Duck Cove sediment is contaminated with Bunker C oil remaining from the 1970 *Arrow* spill.

| Sample | Depth (cm) | PEOM (μg/g) | "Oil" (μg/g) | *n*-alkanes (ng/g) |
|---|---|---|---|---|
| Petpeswick | 0–5 | 15–96 | 1–5 | 0–11 |
| Black Duck Cove | 0–5 | 260–1004 | 25–1292 | 13–31 |
| | 15–20 | 8–10 | 9–12 | 2 |

TABLE 5. Weight range and chemistry of *Arenicola marina* collected from Petpeswick Inlet and Black Duck Cove lagoon, N.S. Weight data from worms used in Experiments 7 and 8. PEOM (pentane-extractable organic matter) and "Oil" (estimates using fluorescence spectroscopy) concentrations determined with six worms collected from each site (during May at Black Duck Cove and August at Petpeswick).

| Origin | Worm wet wt (g) | PEOM[a] (mg/g) | "Oil" (μg/g) |
|---|---|---|---|
| Petpeswick | 2.0–6.6 | 5.0–10.5 | 5–8 |
| Black Duck Cove | 3.2–9.8 | 12.7–14.8 | 9–24 |

[a]After saponification.

in the central portion of the lagoon to a depth of 15–20 cm (Table 4, Fig. 2 and 3).

Worms from both locations were visually indistinguishable. Those collected from Black Duck Cove had a slightly higher weight range (Table 5). The fluorescence data suggest slightly higher levels of aromatic compounds in Black Duck Cove worms; this was also apparent in the SEES. The $f_1$ fractions of worms from both locations were dominated by non-*n*-alkane peaks having retention indices between about 1700 and 2400 (Fig. 4) and there were no apparent qualitative or quantitative differences. The chromatograms of $f_2$ fractions (Fig. 4) were also indistinguishable. No UCM's were visible in worm chromatograms from either location. Population densities were 1–10 and 10–25/m$^2$ at Petpeswick and Black Duck Cove, respectively.

## Results

### EFFECT OF SEDIMENT-BOUND OIL ON WORM BURROWING, SURVIVAL, AND WEIGHT

At the start of all experiments, regardless of oil type and concentration, worms burrowed into sediment within minutes. Cast production usually started about 3–4 h later. With the exception of Experiment 8, worms in control (unoiled) sediment maintained their normal position in the sediment and produced casts during the entire experimental period. For some unknown reason,

TABLE 6. Effect of type and concentration of sediment-bound oil on the sediment-working rate of *Arenicola marina* (oil at all depths of experimental beakers). Each rate represents the mean of 20 observations (5-d experiments with four replicates).

| Expt | Mean sediment oil concn ($\mu$g/g) | Mean sediment-working rate (g sed·g worm$^{-1}$·day$^{-1}$) Control | N2F | SLC | VBC | KC | Mean oiled | % mean control |
|---|---|---|---|---|---|---|---|---|
| 1 | 37 | 7.36 | 2.63 | 2.85 | 4.85 | 4.46 | 3.70 | 54 |
| 2 | 87 | 6.74 | 1.14 | 0.98 | 2.81 | 4.57 | 2.38 | 35 |
| 3 | 153 | 1.49 | 0.62 | 3.08 | 0.23 | 5.04 | 2.24 | 33 |
| 4 | 182 | 6.11 | 1.05 | 1.55 | 1.38 | 2.32 | 1.58 | 26 |
| 5 | 275 | 12.41 | 0.76 | 0.52 | 0.23 | 0.28 | 0.45 | 7 |
| Mean | | 6.82 | 1.24 | 1.80 | 1.90 | 3.33 | | |
| % mean control | | | 18 | 26 | 28 | 49 | | |
| % aromatics in oil[a] | | | 9.2 | 0.9 | 4.3 | 0.6 | | |

[a]Data from Anderson et al. (1974).

the working rates of control worms in Experiment 8 were very low and some stopped producing casts before the end of the experiment.

In Experiments 1–5 (oil at all depths in experimental beakers) some worms surfaced when oil concentrations were as low as 153 $\mu$g/g and some worms died (in sediment and on surface) when concentrations reached 275 $\mu$g/g. In Experiment 6 (oil in surface layer only), surfacing and death of worms occurred when oil concentrations in the surface layer exceeded 600 $\mu$g/g. In all experiments, only 14% of the worms died (mostly in Experiments 5 and 8). There are insufficient data to rank the oils used according to their toxicity.

Control worms and worms exposed to South Louisiana crude (SLC) and Kuwait crude (KC) did not experience any appreciable weight changes during experiments, while the wet weights of worms exposed to No. 2 fuel (N2F) and Venezuelan Bunker C (VBC) dropped on the average of 8 and 16%, respectively.

### Effect of Sediment-Bound Oil on Sediment-Working Rate

In all experiments, the daily sediment-working rate of *Arenicola* gradually increased during the first few days, presumably reflecting the adaptation of worms to their new environment.

The comparative effect of the four API reference oils on sediment working rate was examined in Experiments 1–5 (Table 6). N2F had the greatest effect (82% depression) and KC the least (51% depression). Except for the transposition of VBC and SLC, which could reflect insufficient data, the effect can generally be related to the aromatic content of the oils. The concentration of sediment-bound oil also affects working rate (Table 6). Sediment-working rate was also markedly reduced when oil was restricted to the surface layer (Table 7), the situation that more closely approximates the natural oiled environment (Table 4).

The possible effect of weathering on an oil's influence on working rate was examined by comparing surface sediment from Black Duck Cove (naturally contaminated with weathered ABC) with Petpeswick sediment spiked with ABC stored in the laboratory for 7 yr (Table 8). The results are inconclusive. The "fresh" ABC had no influence on sediment-working rate in the concentration range of 95–190 $\mu$g/g. This reflects either a much lower toxicity of fresh ABC compared with the VBC from the API reference collection (Table 6) or the loss of toxic constituents from ABC during storage in the laboratory.

As also observed for other deposit-feeding organisms (Rhoads 1963; Gordon 1966), temperature has a pronounced effect on sediment-working rate of *Arenicola*. In Experiment 9, the working rate was greatest at 12–15°C, averaging 4.6 g/g worm per d. At higher and lower temperatures (Table 1), the working rate averaged 1 g/g worm per d or less. Cadée (1976) has determined the seasonal variations in cast production by *Arenicola* populations in the Dutch Wadden Sea and these as well appear to be closely related to environmental temperature.

TABLE 7. Effect of oil stratification on mean sediment working rate by *Arenicola marina* (Expt 6). The upper 8 cm of sediment contained Venezuelan Bunker C, the lower 8 cm contained no oil at the beginning of the experiment but trace levels at the end. Each rate is the mean of 20 observations.

| Surface sediment oil concn ($\mu$g/g) | Mean sediment-working rate (g sed·g worm$^{-1}$·day$^{-1}$) | % mean control |
|---|---|---|
| Control | 14.73 | — |
| 79 | 1.75 | 12 |
| 338 | 3.40 | 23 |
| 617 | 4.30 | 29 |
| 2086 | 2.62 | 18 |

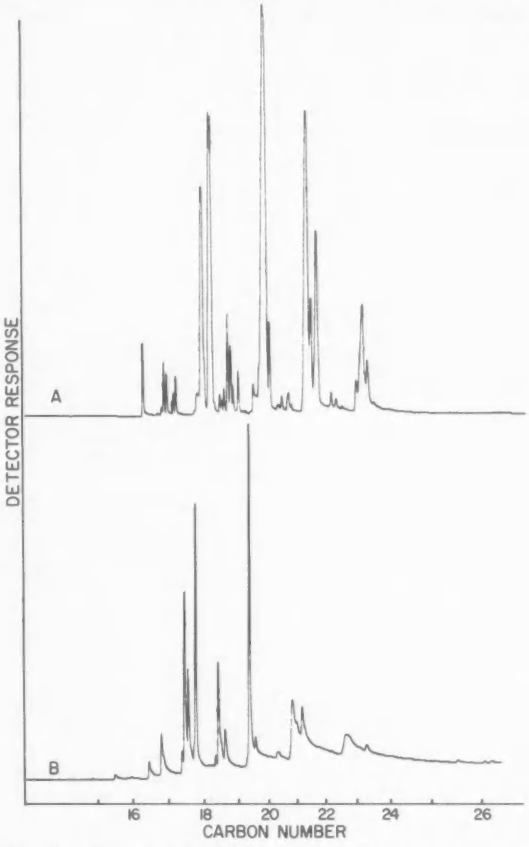


FIG. 4. Characteristic chromatograms of *A. marina*. A, $f_1$ fraction, 2 $\mu$L, atten. 128; B, $f_2$ fraction, 5 $\mu$L, atten. 32.

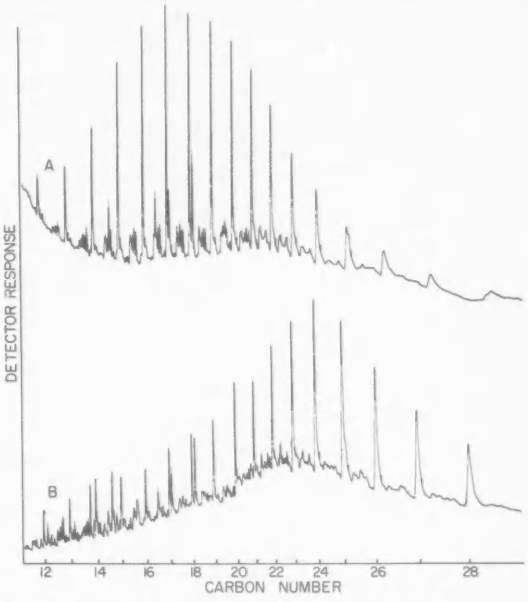


FIG. 5. Removal of *n*-alkanes and isoprenoids from sediment as a result of working by *A. marina*. Both chromatograms from Expt 2, $f_1$. A, Petpeswick sediment containing KC, 2 $\mu$L, atten. 4; B, casts, 20 $\mu$L, atten. 8.

## REMOVAL OF HYDROCARBONS FROM SEDIMENT DURING THE WORKING PROCESS

Removal of the four API reference oils from sediment during the working process is illustrated in Table 9. These results were obtained from experiments conducted with unstratified sediment, so there is no possibility that the results could be influenced by the ingestion of oil-free sediment. No data are available from Experiments 4 and 5 because, due to higher oil concentrations, cast production on the final day of the experiments was negligible.

The loss of total oil as measured gravimetrically (PEOM) was quite variable, ranging between 19 and 94%. Despite the variability, concentrations in casts were always lower than in sediment. The smallest losses were associated with VBC. The mean loss for all oils in

TABLE 8. Effect of oil weathering upon mean sediment-working rates by *Arenicola marina*. ABC is *Arrow* Bunker C oil that has been stored in a can (opened several times) at room temperature for 7 yr. Experiment 8 was stratified and the deep sediment in experimental beakers came from Petpeswick (0–5 cm). Mean sediment-working rates are based on 120 and 112 observations for Expt 7 and 8, respectively.

| Expt | Sediment oil type | Mean oil concn ($\mu$g/g) | Mean sediment-working rate (g sed·g worm$^{-1}$·day$^{-1}$) | % mean control |
|---|---|---|---|---|
| 7 | Petpeswick (Control) | 0 | 10.6 | |
| | Petpeswick plus ABC | 190 | 10.1 | 95 |
| | Black Duck Cove (0–5 cm) | 800 | 2.5 | 24 |
| | Black Duck Cove (15–20 cm) | trace | 5.9 | 56 |
| 8 | Petpeswick (Control) | 0 | 1.7 | |
| | Petpeswick plus ABC | 95 | 2.1 | 124 |
| | Black Duck Cove (0–5 cm) | 260 | 5.0 | 294 |

TABLE 9. Removal of petroleum hydrocarbons from sediment as a result of sediment working by *Arenicola marina*. All concentrations are corrected for background levels in control sediment. Because cast production on the final day of experiments was negligible for N2F in Expt 2 and N2F and VBC in Expt 3, chemical analyses could not be conducted. The $f_1$ fraction contains *n*-alkanes, cycloalkanes, isoprenoids, some alkenes; the $f_2$ fraction contains some alkenes polycyclic aromatics; and the $f_3$ fraction contains high molecular weight PAH's.

| Expt | Oil type | Sample | Total oil (PEOM) (μg/g) | Oil (PEOM) (μg/g) | | | *n*-Alkanes and isoprenoids (μg/g) | "Oil" (μg/g) |
|---|---|---|---|---|---|---|---|---|
| | | | | $f_1$ | $f_2$ | $f_3$ | | |
| 1 | N2F | Sediment | 37.7 | 6.1 | 5.2 | 0.0 | 3.20 | 16.4 |
| | | Cast | 14.0 | 0.0 | 3.0 | 0.0 | 0.06 | 3.9 |
| | | Δ | 23.7 (63%) | 6.1 (100%) | 2.2 (42%) | 0.0 | 3.14 (98%) | 12.5 (76%) |
| | VBC | Sediment | 24.4 | 2.3 | 5.6 | 6.3 | 0.41 | 25.8 |
| | | Cast | 19.7 | 1.5 | 3.2 | 6.3 | 0.06 | 23.3 |
| | | Δ | 4.7 (19%) | 0.8 (35%) | 2.4 (43%) | 0.0 | 0.35 (86%) | 2.5 (10%) |
| | SLC | Sediment | 46.0 | 10.8 | 4.3 | 5.5 | 1.89 | 51.3 |
| | | Cast | 14.5 | 6.2 | 4.6 | 0.0 | 0.47 | 22.7 |
| | | Δ | 31.5 (68%) | 4.6 (43%) | GAIN | 5.5 (100%) | 1.42 (75%) | 28.6 (56%) |
| | KC | Sediment | 40.9 | 16.4 | 7.0 | 5.2 | 1.56 | 28.3 |
| | | Cast | 24.7 | 4.4 | 3.4 | 0.0 | 0.17 | 7.6 |
| | | Δ | 16.2 (40%) | 12.0 (73%) | 3.6 (51%) | 5.2 (100%) | 1.39 (89%) | 70.7 (73%) |
| 2 | VBC | Sediment | 58.5 | 13.7 | 16.8 | 14.1 | 0.47 | 38.8 |
| | | Cast | 43.8 | 11.9 | 16.7 | 5.3 | 0.38 | 26.4 |
| | | Δ | 14.7 (25%) | 1.8 (13%) | 0.1 (1%) | 8.8 (62%) | 0.09 (20%) | 12.4 (32%) |
| | SLC | Sediment | 95.3 | 26.2 | 9.8 | 6.3 | 1.73 | 136.5 |
| | | Cast | 6.0 | 6.1 | 0.0 | 8.1 | 0.57 | 34.7 |
| | | Δ | 89.3 (94%) | 20.1 (77%) | 9.8 (100%) | GAIN | 1.16 (67%) | 100.8 (74%) |
| | KC | Sediment | 86.3 | 27.1 | 26.1 | 7.3 | 2.05 | 188.1 |
| | | Cast | 30.7 | 5.5 | 8.7 | 1.8 | 0.40 | 122.3 |
| | | Δ | 55.6 (64%) | 21.6 (80%) | 17.4 (67%) | 5.5 (75%) | 1.65 (80%) | 65.8 (35%) |
| 3 | SLC | Sediment | 98.3 | 37.8 | 12.2 | 2.0 | 5.57 | 140.3 |
| | | Cast | 38.1 | 25.2 | 2.2 | 1.4 | 1.63 | 52.9 |
| | | Δ | 60.2 (61%) | 12.6 (33%) | 10.0 (82%) | 0.6 (30%) | 3.94 (71%) | 87.4 (62%) |
| | KC | Sediment | 110.9 | 35.2 | 30.4 | 5.2 | 6.62 | 101.1 |
| | | Cast | 56.6 | 18.1 | 16.1 | 10.1 | 2.76 | 74.1 |
| | | Δ | 54.3 (49%) | 17.1 (49%) | 14.3 (47%) | GAIN | 3.86 (58%) | 27.0 (27%) |
| Overall mean | | Sediment | 66.5 | 19.5 | 13.0 | 5.8 | 2.61 | 80.6 |
| | | Cast | 27.6 | 8.8 | 6.4 | 3.7 | 0.72 | 40.9 |
| | | Δ | 38.9 (58%) | 10.7 (55%) | 6.6 (51%) | 2.1 (36%) | 1.89 (72%) | 39.7 (49%) |

these experiments was 58% or 38.9 μg/g. Due to the smaller sample sizes, the variability in PEOM data after column chromatography was even greater. The mean losses were 55, 51, and 36% for the $f_1$ (e.g. *n*-alkanes, cycloalkanes, isoprenoids, some alkenes), $f_2$ (e.g. some alkenes, polycyclic aromatics), and $f_3$ (e.g. high molecular weight PAH's) fractions, respectively.

The removal of *n*-alkanes and isoprenoids ranged from 20 to 98%, averaging 72% or almost 2 μg/g. Losses were proportional to the *n*-paraffin content of the individual oils (Anderson et al. 1974). Generally speaking, the shorter the chain length, the greater the loss (Fig. 5). The isoprenoids pristane and phytane were lost at a lesser rate than straight-chain alkanes as demonstrated by lower $C_{17}$/pristane and $C_{18}$/phytane ratios in casts (Table 10). The pristane/phytane ratio was also lower in casts.

TABLE 10. Mean ratios observed in oiled sediment and casts produced by *Arenicola marina* (Expt 1–3).

| Sample | $C_{17}$/pristane | $C_{18}$/phytane | Pristane/phytane |
|---|---|---|---|
| Sediment | 2.10 | 2.38 | 1.69 |
| Cast | 1.70 | 1.69 | 1.28 |

Oil concentrations, as estimated by fluorescence spectroscopy (which detects only aromatic compounds), dropped between 10 and 76%, averaging 49% or 39.7 μg/g. Comparison of SEES's of sediment and casts indicates that aromatic hydrocarbons of all ring sizes are being removed (Fig. 6).

The removal of hydrocarbons from Black Duck Cove surface sediment containing weathered Bunker C is illustrated in Table 11. Although the loss percentages are similar to those observed with the fresh API oils (except for the $f_1$ fraction) (Table 9), the absolute losses of most components were about an order of magnitude higher. This difference probably reflects the much lower

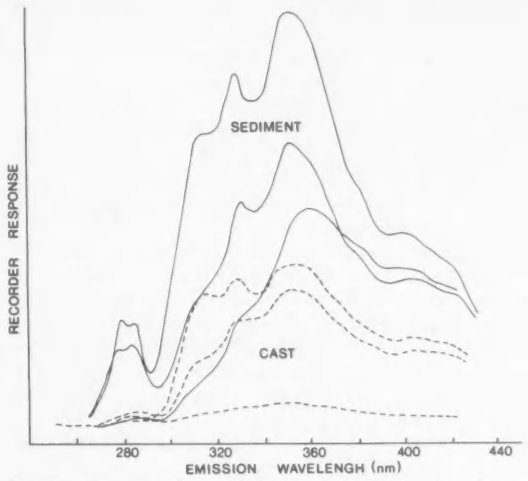


FIG. 6. Changes in synchronous excitation of emission spectra (SEES) produced by sediment working by *A. marina*. Sediment was spiked with SLC (Expt 1–3).

TABLE 11. Removal of hydrocarbons as a result of sediment working by *Arenicola marina* from Black Duck Cove sediment, which contains weathered Bunker C remaining from the 1970 *Arrow* spill. Combined data from Expt 7 and 8. The $f_1$ fraction contains *n*-alkanes, cycloalkanes, isoprenoids, some alkenes; the $f_2$ fraction contains some alkenes, polycyclic aromatics; and the $f_3$ fraction contains high molecular weight PAH's.

| | Total oil (PEOM) (μg/g) | Oil (PEOM) (μg/g) | | | "Oil" (μg/g) |
|---|---|---|---|---|---|
| | | $f_1$ | $f_2$ | $f_3$ | |
| Sediment | 1004.1 | 91.4 | 59.7 | 25.4 | 946.2 |
| | 607.6 | 57.2 | 31.0 | 10.6 | 511.5 |
| | 360.7 | 94.2 | 42.1 | 46.0 | 1291.7 |
| Mean | 657.5 | 81.0 | 70.4 | 27.3 | 916.4 |
| Cast | 125.0 | 19.5 | 6.3 | 2.1 | 65.1 |
| | 240.4 | 66.0 | 35.6 | 13.5 | 51.5 |
| | 378.8 | 116.9 | 41.7 | 48.4 | 1140.0 |
| Mean | 248.1 | 67.5 | 27.9 | 21.3 | 420.2 |
| Difference | 409.4 | 13.5 | 42.5 | 6.0 | 496.2 |
| % removed | 62 | 17 | 60 | 22 | 54 |

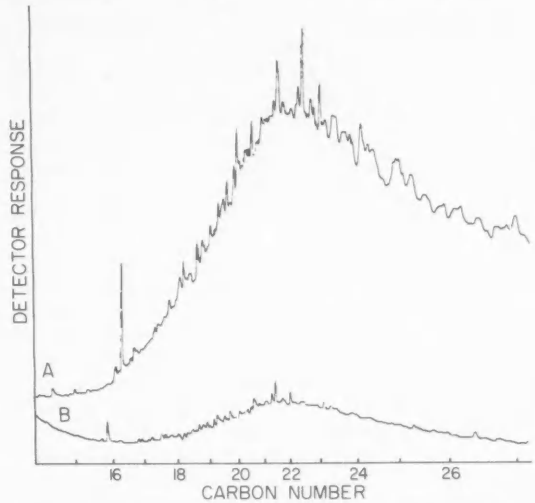


FIG. 7. Removal of the unresolved complex mixture (UCM) from Black Duck Cove surface sediment as a result of working by *A. marina*, Expt 8. A, initial sediment, 1 μL, atten. 32; B, cast, 2 μL, atten. 64.

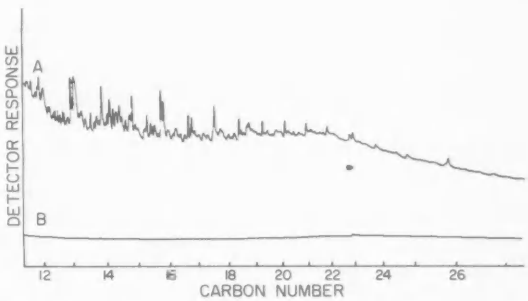


FIG. 8. Removal of $f_2$ fraction hydrocarbons from sediment spiked with SLC as a result of working by *A. marina*, Expt 3. A, initial sediment, 5 μL, atten. 128; B, cast, 10 μL, atten. 256.

toxicity of the weathered ABC compared with the fresh API oils, which enables worms to work sediment with considerably greater oil concentrations.

The unresolved complex mixture (UCM) in the four API reference oils and the fresh ABC was minor and there was no apparent difference between sediment and casts (e.g. Fig. 5). There is, however, a large UCM in the $f_1$ fraction of Black Duck Cove surface sediment which was almost completely removed during working (Fig. 7). No quantitative data are available on the loss of the UCM (presumably composed principally of cycloalkanes), but visual inspection suggests that the loss is of the order of that observed for *n*-alkanes and isoprenoids (72%, Table 9).

No quantitative GLC data are available for the $f_2$ fraction in sediments and casts. However, comparison of chromatograms (e.g. Fig. 8) indicates that hydrocarbons from this fraction are being removed from sediment, as was also indicated in the gravimetric data (Table 9).

Reviewing all chemical data from Experiments 7–9, the amounts of petroleum-derived hydrocarbons removed from sediment worked by Black Duck Cove worms were no different than the amounts removed from sediment worked by worms from Petpeswick.

TABLE 12. Net uptake of petroleum hydrocarbons by unoiled Petpeswick worms exposed to the four API reference oils for 5 d.

| Expt | Oil | PEOM (mg/g)[a] Total | $f_1$ | $f_2$ | $f_3$ | "Oil" (mg/g) |
|---|---|---|---|---|---|---|
| 1 | Control | 6.32 | 0.19 | 0.02 | 0.00 | 0.010 |
| | N2F | 7.00 | 0.26 | 0.08 | 0.00 | 0.250 |
| | VBC | 12.85 | 1.17 | 0.08 | 0.08 | 0.059 |
| | SLC | 9.09 | 0.55 | 0.24 | 0.06 | 0.189 |
| | KC | 6.98 | 0.48 | 0.05 | 0.03 | 0.085 |
| 2 | Control | 8.31 | 0.20 | 0.04 | 0.02 | 0.009 |
| | N2F | 9.84 | 0.60 | 0.04 | 0.01 | 0.166 |
| | VBC | 11.84 | 0.60 | 0.11 | 0.02 | 0.575 |
| | SLC | 10.33 | 0.35 | 0.01 | 0.01 | 0.360 |
| | KC | 6.03 | 0.41 | 0.02 | 0.00 | 0.642 |
| 6 | Control | 4.91 | 0.71 | 0.47 | 0.24 | 0.024 |
| | VBC | 7.53 | 1.03 | 1.80 | 0.36 | 0.122 |
| | VBC | 6.40 | 1.57 | 0.49 | 0.05 | 0.312 |
| | VBC | 5.29 | 2.36 | 0.12 | 0.02 | 0.237 |
| | VBC | 7.20 | 1.72 | 0.14 | 0.03 | 0.465 |
| Mean oiled | | 8.37 | 0.93 | 0.27 | 0.06 | 0.289 |
| Mean control | | 6.51 | 0.37 | 0.18 | 0.07 | 0.014 |
| Uptake | | 1.86 | 0.56 | 0.09 | 0.00 | 0.275 |

[a]After saponification.

### UPTAKE OF OIL BY WORMS

Some uptake of oil by worms during experiments was apparent (Table 12). The gravimetric data indicated a mean net oil uptake of almost 2 mg/g (wet weight) during the 5-d experiments (not including hydrocarbons possibly lost during the 24-h flushing period). Most of the uptake appears to be from a hydrocarbon fraction that does not pass through the silica-gel – alumina column. Of the three fractions recovered, uptake was greatest from the $f_1$ fraction. The fluorescence data also indicated uptake. There was no visible uptake of hydrocarbons from either the $f_1$ or $f_2$ fractions during the experiments seen in the gas chromatography data. Any uptake occurring is masked by the high and variable concentration of naturally occurring hydrocarbons present in the worms (Fig. 4).

## Discussion

Since the oil concentrations used in these experiments were intentionally low, in the sublethal range, few worms died and therefore sufficient data are not available to rank the API reference oils according to their toxicity. However, on the basis of changes in worm weight and in sediment-working rate, the order of decreasing effect on *Arenicola* appears to be N2F, VBC, SLC, and KC. An identical sequence has been reported for the toxicity of water-soluble fractions of the same oils to six other species of polychaete worms (Rossi et al. 1976; Carr and Reish 1977) and six species of fish and crustaceans (Anderson et al. 1974). Because our experiments were conducted using oiled-sediment and not water-soluble fractions, it is not possible to compare the sensitivity of *Arenicola* with that of other marine organisms tested. The toxicity of the four API reference oils is generally assumed to be related to their aromatic content and it is believed that most of the toxicity observed is produced by the naphthalene fraction (Rossi et al. 1976). Some of the effects on *Arenicola* observed could have been caused by hydrocarbons brought into burrows with ventilating water. Levell (1976) observed similar decreases in working activity when natural populations of *Arenicola* were sprayed with Kuwait crude oil at a rate of 0.2 L/m$^2$. There was no significant difference in the average amount of sediment worked by worms from Petpeswick and Black Duck Cove (Experiments 7 and 8, over 400 comparisons), suggesting that Black Duck Cove worms do not have a different tolerance to oil because of exposure to weathering Bunker C during their entire lives.

The data obtained indicate that during the process of sediment working by *Arenicola* substantial amounts of petroleum hydrocarbons are removed from the sediment (Tables 9 and 11, Fig. 5–8). While all hydrocarbon classes are affected, the removal of aliphatic hydrocarbons appears to be greater than that of aromatics. As discussed in our preliminary report (Prouse and Gordon 1976), there are four processes that could be operating to produce the lower hydrocarbon concentrations consistently observed in casts: (1) sediment sorting during feeding, (2) dissolution, (3) assimilation, and (4) biodegradation.

Hylleberg (1975) and Cadée (1976) report that *Arenicola* selects sediment for ingestion, preferring particle sizes generally $<500$ μm. *Arenicola* cannot ingest particles $\gtrsim 2$ mm. Since Petpeswick sediment is fine and well sorted, very little sorting occurred when *Arenicola* fed upon it (Table 3). Therefore it is highly unlikely that the lower hydrocarbon concentrations observed in casts of worms used in experiments employing Petpeswick sediment represent an artifact produced by sediment selection. There was, however, considerable sorting when *Arenicola* fed on Black Duck Cove sediment, which is 24% gravel. However, the selection of finer sediment should increase, not decrease, the effective concentration of ingested oil, since sediment-bound organic matter has a tendency to associate with the finer size fractions. We therefore dismiss sediment sorting as an explanation for the hydrocarbon loss consistently observed.

The entire sediment-working process (filling of headshaft, ingestion, digestion, and defecation) takes place in the presence of seawater. Therefore loss by dissolution is possible, especially with the more soluble aromatic hydrocarbons. We have no data to quantify the potential importance of this process, so it should be considered as possibly responsible for the removal of some aromatic compounds.

Although not apparent in the GLC data, perhaps

TABLE 13. Theoretical rates of oil removal by *Arenicola* populations.

| | Fresh API oils (Expt 1–3) | Weathered Bunker C (Expt 7&8) |
|---|---|---|
| Oil concn in sediment (μg/g) | 67 | 658 |
| Oil removed during working (μg/g) | 39 | 409 |
| Daily working rate (g/worm) | 13.86 | 18.51 |
| Est annual avg daily working rate (after tidal and temp correction) (g/worm) | 10 | 10 |
| Annual oil removal (g/worm) | 0.142 | 1.49 |
| Total oil in $m^2$ to depth of 5 cm (g)[a] | 6.517 | 64.435 |
| Yr to remove[b] oil present at worm density of | | |
| $1/m^2$ | 45.9 | 43.2 |
| $10/m^2$ | 4.6 | 4.3 |
| $25/m^2$ | 2.0 | 1.7 |
| $50/m^2$ | 1.0 | 0.9 |
| $100/m^2$ | 0.5 | 0.4 |

[a]Assuming uniform concentration.
[b]Assuming all components removed at same rate.

because of the high background of biogenic hydrocarbons (Fig. 4), uptake of hydrocarbons into worm tissue did occur during the 5-d experiments (Table 12). Uptake of hydrocarbons by other polychaete worms has been reported (*Neanthes*, Rossi et al. 1976; and *Phascolosoma*, Anderson et al. 1977). Concentrations are generally in the low parts per million range and there is a marked preference for aromatics (Rossi et al. 1976; Neff et al. 1976; Rossi 1977). Rossi (1977) also reports that most of the aromatic hydrocarbons accumulated by *Neanthes* are derived from water and not from sediment.

The importance of uptake of hydrocarbons into worm tissue is evident by comparing it with the loss of hydrocarbons from sediment worked by the worms. Using the mean net uptake of total oil, which does not include oil lost during the 24-h flushing period at the end of experiments, and the average worm weight (5 g wet), we calculate the daily uptake rate to be 1.86 mg/worm (assuming no metabolism, which may not be true; Lee 1977; Rossi 1977). Multiplying the mean removal rate of total oil (Table 9) by the mean daily working rate of Experiments 1–3 (15 g/worm) yields a daily removal rate of 584 μg/worm. This is only one third the measured uptake. This discrepancy could be caused by two factors. First, the mean uptake rate for total oil could be in error. Only a limited number of worms were analyzed (30) and the results were quite variable. Also the uptake, as measured by PEOM, was only an average of 30% of the background PEOM concentrations in worms. Second, a large portion of the hydrocarbons observed in the worms could have originated from water pumped into the burrows for ventilation and not from ingested sediment. In summary, uptake of oil by *Arenicola* can account for the removal of some of the oil lost from sediment during the working process.

Biodegradation by microorganisms also appears responsible for some of the hydrocarbon removal observed. The preferential removal of lower molecular weight *n*-paraffin and isoprenoid homologs (Fig. 5) is characteristic of microbial degradation, as is the preferential removal of straight-chain hydrocarbons compared with branched (Table 10) (Mechalas et al. 1973). Biodegradation may also be responsible for some of the loss of aromatic hydrocarbons (Gibson 1977). In view of what is known about the feeding habits of lugworms, the loss of oil by biodegradation seems quite reasonable. Hylleberg (1975) reported that high concentrations of microorganisms, including bacteria, are present in the sand at the bottom of the headshaft (Fig. 1). Their growth is stimulated by nutrients present in the surrounding reduced sediments and by oxygen present in the irrigation water. Excretions from the worms may also stimulate microbial growth. Hylleberg (1975) proposed a gardening concept whereby the worm enriches its potential food supply by stimulating the growth of digestible microorganisms in the sediment it is ingesting. Biodegradation may also be substantial in the casts after deposition. Hylleberg (1975) reports that many bacteria are passed through the gut of lugworms undigested, and growth conditions in the casts should be highly favorable.

The possible impact of *Arenicola* on the weathering of oil in marine sediments (by uptake, stimulated microbial degradation, and possibly dissolution) is illustrated by calculating the theoretical time required to remove all the oil present in a square metre of sediment using the data generated in these experiments (Table 13). The daily working rates measured were corrected for tidal and seasonal temperature effects (Cadée 1976) and the estimate of 10 g/worm represents the daily working rate averaged over an entire year. The results indicate that with the fresh API and weathered Bunker C oils a population density of 10 worms/$m^2$ would in theory remove all oil present in about 4 yr. A density of 10–25 worms/$m^2$, such as found in Black Duck Cove, would require 2–4 yr to remove all the oil. These calculations, however, must be interpreted with caution because they assume that all oil components are removed at an equal and constant rate and that the rate of removal is not a function of oil concentration. For example, Rashid (1974) reports an increase of resins and NSO (nitrogen, sulfur, and oxygen) compounds during the weathering of *Arrow* Bunker C oil. They do however illustrate the important influence that *Arenicola*, and presumably other deposit-feeding organisms, can have on the weathering rate of sediment-bound oil if the concentrations are in the range that the worm can tolerate.

The lack of high levels of petroleum-derived hydrocarbons in Black Duck Cove worms, despite the fact that they have lived all their lives in sediment containing weathered Bunker C oil, is in agreement with the results of other recent investigations. Anderson et al. (1977) and Rossi (1977) have shown that other poly-

chaete worms (*Phascolosoma* and *Neanthes*, respectively) accumulate very few aromatic hydrocarbons from ingested sediment and that those accumulated can be cleared rapidly. Rossi (1977) and Lee (1977) also present evidence that aromatic hydrocarbons accumulated by polychaetes can be metabolized. Our results support the concept that bioaccumulation of hydrocarbons in worms does not appear to be significant, as opposed to molluscs for example, even in the case of worms exposed to persistent oil spills.

With the information at hand, it is possible to reconstruct the probable events that affected the *Arenicola* population in the lagoon at Black Duck Cove at the time of the *Arrow* spill and during the period between the spill and summer 1976 when our observations began. It is assumed that *Arenicola* was present in the lagoon at the time of the spill. Unfortunately, no detailed biological observations were made at this site during the Operation Oil exercise (Anon. 1970).

Most of the oil entering the lagoon at the time of the spill was deposited in the upper portion of the intertidal zone; very little was deposited initially on the lagoon floor which is covered at low tide with 10–50 cm of water (Owens and Rashid 1976). Judging from the results of Levell (1976), mortality of *Arenicola*, and presumably other organisms living in the upper intertidal zone, was probably immediate and very heavy, if not complete. In contrast, mortality in the lower portion of the intertidal zone was probably less and somewhat delayed, since the oil reaching it was probably derived from that stranded above (Vandermeulen and Gordon 1976). Much of the mortality could have been the result of sublethal effects, for example working rates being reduced to a level that prevented worms from obtaining sufficient food. In any event, an unknown portion of the worms, most likely on the lagoon floor, probably survived the oiling incident.

During the first few years after the spill, there was probably little recolonization of the upper intertidal zone from below because of continuing high levels of relatively fresh oil in sediment, as observed by Levell (1976). Reestablishment probably began when weathering processes reduced the toxicity of sediment to the level that worms could tolerate. The working rates of the first worms to recolonize heavily oiled sediment were undoubtedly reduced quite substantially (Table 6). However, due to the presence of the worms, the weathering rate of sediment-bound oil increased (Table 13), reducing the toxicity further. As of 1977 the weathered oil in Black Duck Cove lagoon sediment does not appear to have a significant effect on sediment-working rate (Table 8) and worms are found in the upper intertidal zone within a metre of the remaining stranded oil pavement. It is noteworthy that the present-day population of *Arenicola* in Black Duck Cove lagoon (10–25/$m^2$) is the densest we are aware of in Nova Scotia. This suggests the possibility that *Arenicola* is more resistant to heavy fuel oils than other deposit-feeding organisms and may represent an opportunistic species.

Since the present population density of *Arenicola* at Black Duck Cove is 10–25 worms/$m^2$, all the oil present should in theory be removed in 2–4 yr (Table 13). The fact that oil is still present in the sediment 7 yr after the spill does not indicate the calculations in Table 13 are unrealistic. As oil is removed from the lower portion of the intertidal zone more is supplied from the stranded oil above. This process will undoubtedly continue until the stranded oil is completely removed or becomes inert, perhaps as long as 150 yr (Vandermeulen and Gordon 1976). It would be interesting to revisit this site in the future and examine the hydrocarbon chemistry of the sediment with gas chromatography–mass spectrometry. If there are hydrocarbon components such as PAH's and NSO compounds that are not being removed by chemical, physical, or biological weathering processes, they should gradually accumulate in the sediments on the floor of the lagoon.

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*SESSION I. Summary and Overview*

# Long-Term Fate of Petroleum Hydrocarbons After Spills — Compositional Changes and Microbial Degradation

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The recovery of cold marine environments from the effects of oil spills has recently become a subject of considerable scientific and public concern because of the many large spills from tanker accidents and because of the hazards inherent in the exploration and production of oil in hostile northern marine environments. Because of the scientific investigations associated with the *Torrey Canyon*, *Florida*, *Arrow*, *Metula*, and other tanker accidents and from the *Bravo* blowout in the North Sea, a great deal of experience (and some quantitative information) concerning the recovery of marine environments has been accumulated. It has been the aim of the Symposium to bring together this scattered body of information to form a unified picture of the existing knowledge.

Session I, which dealt with the long-term changes in the composition of oil when it is exposed to the marine environment, clearly demonstrated that the potential for recovery exists but may take many years. The rate of recovery depends on the type and amount of oil, but more critically upon energy needed to drive the complex series of physical, chemical, biological, and geological processes that disperse and degrade the oil.

The single most important type of energy is provided by wind and waves at the time of the spill. Immediately on entering the marine environment, oil tends to spread out on the surface of the water. Sufficient wave action breaks up the oil slick into droplets and disperses them into the water column, where they can be swept away by the currents. This is a natural application of the old sanitary engineering motto, "The solution to pollution is dilution." This simple mechanical dispersion was so effective during the *Bravo* blowout in the North Sea that oil in the water column was reduced to barely detectable levels within 2 mo after the blowout was brought under control (Mackie et al.; Grahl-Nielsen et al.).

Mechanical energy from wave action is even more important in the removal of stranded oil from fouled shorelines. For example, natural cleaning by moving sand grains on high energy beaches may be virtually complete within a few weeks, whereas in sheltered low-energy areas the oil may become embedded in the sediments (Owens), where it may persist for many years, particularly if the sediments are anoxic (Teal et al.; Gassmann and Gunkel[1]; Mayo et al.). Similarly, in the quiescent areas among floating ice, wave action may not be sufficient to bring about physical dispersion, and oil on beaches may be completely protected from weathering by ice cover (Owens).

Although the importance of mechanical processes during initial stages of weathering has long been recognized, their role in subsequent stages has taken on a new dimension as a consequence of Session I.

The second physical process, evaporation, removes the more volatile hydrocarbons from spilled oil. In the *Bravo* incident, where oil at 75–90°C was ejected some 50 m into the atmosphere, evaporation may have prevented it from entering the aqueous environment at all (Mackie et al.). At the other extreme, evaporation is so severely hampered under arctic ice that it has no significant effect on the oil (Atlas et al.).

Although the physical processes of dispersion and evaporation may effectively reduce the concentration or alter the composition of oil in the vicinity of a spill, they do not directly degrade it. Nevertheless, dispersion greatly increases the surface area of the oil and thereby renders it more susceptible to chemical and biological degradation.

For many years it has been accepted that attack by microorganisms is the most significant process for degrading oil to carbon dioxide. A background population of oil-degrading bacteria, yeasts, and fungi is present in all marine environments (Stewart and Marks), and slow but measurable degradation occurs even under arctic conditions (Stewart and Marks; Atlas et al.). However, few organisms can attack the entire spectrum of hydrocarbons and heterocyclic compounds present in petroleum, and a diverse population is needed. The rate and extent of biodegradation is often limited by the supply of nutrients (Stewart and Marks), and Session I suggested that biodegradation might not be as rapid and important a process for eliminating petroleum from cold marine environments as had previously been believed. For example, biodegradation was reported to

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be "slow" (time scale of years) in a variety of intertidal environments ranging from exposed rocky Pacific coasts (Cretney et al.) to sheltered Atlantic lagoons, salt marshes, harbors, and estuaries (Teal et al.; Keizer et al.; Mayo et al.; Van Vleet and Quinn) and at a European oil terminal (Gassmann and Gunkel[1]).

Wherever environmental biodegradation has been studied, the alkanes tend to be most susceptible, and substituted polynuclear aromatic hydrocarbons the most resistant (Teal et al.; Mackie et al.; Grahl-Nielsen et al.). When oil is buried in sediments, the oxygen supply is often the rate-controlling factor for degradation. In some anoxic sediments, hydrocarbons were still present 7 yr after a spill (Teal et al.).

This session further emphasized that caution must be exercised in extrapolating laboratory biodegradation data to the environment, and that field observations must be interpreted carefully to avoid ambiguities arising from additional new imputs of oil to the study area (Mayo et al.; Keizer et al.). In addition, it was pointed out that the concept of a "half-life" for the removal of oil from the environment analogous to that for radioactive decay was inappropriate since the kinetics of the two processes are entirely different.

The session did not consider photochemical reactions or the intake and excretion of petroleum by macrofauna; and only dealt very briefly with the sedimentation of oil from the water column (Van Vleet and Quinn). Both these processes may play a significant role in the recovery of marine ecosystems from oil spills.

In conclusion, the information presented in Session I suggested that every spill incident studied thus far is nearly unique. Information regarding the recovery of one environment may provide insight into processes in another, but direct analogy or extrapolation is dangerous. The time scale for the disappearance of petroleum and the recovery of the ecosystem depends primarily on the availability of adequate mechanical energy for dispersion and chemical energy for biodegradation. Under favorable conditions (North Sea offshore platform) recovery may be in a fairly advanced stage after less than a year. Under unfavorable conditions (West Falmouth) recovery may take longer than 10 yr.

Nevertheless, cautious predictions of the course of events following a spill may be made provided the correct environmental biological and chemical data are available. To improve our capabilities for prediction we need long-term studies in more detail, in more widely varied environments; we need to coordinate chemical analyses for hydrocarbons with microbiological studies; and we need better quantitative theories describing evaporation, dispersion, and photochemical degradation, expecially in high-energy environments.

**Symposium on Recovery Potential of Oiled Marine Northern Environments**

*SESSION II*

## Physiological Stresses and Response in Chronically Oiled Organisms

CHAIRMEN: J. W. ANDERSON AND D. C. MALINS

# Uptake of Hydrocarbons from Marine Sediments Contaminated with Prudhoe Bay Crude Oil: Influence of Feeding Type of Test Species and Availability of Polycyclic Aromatic Hydrocarbons[1]

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ROESIJADI, G., J. W. ANDERSON, AND J. W. BLAYLOCK. 1978. Uptake of hydrocarbons from marine sediments contaminated with Prudhoe Bay crude oil: influence of feeding type of test species and availability of polycyclic aromatic hydrocarbons. J. Fish. Res. Board. Can. 35: 608–614.

Selected benthic animals were exposed to marine sediments contaminated with Prudhoe Bay crude oil, and uptake of hydrocarbons was monitored under various experimental schemes. When uptake of aliphatic and diaromatic hydrocarbons by two deposit feeders, *Macoma inquinata* and *Phascolosoma agassizii*, was compared with that of a suspension feeder, *Protothaca staminea*, we found that the deposit feeders generally accumulated hydrocarbons to a greater extent than the suspension feeder. However, other factors, such as the intrinsic capabilities of species to accumulate hydrocarbons, also played an important role in the extent of contamination. Hydrocarbon concentrations in the bivalve species (*M. inquinata* and *P. staminea*) increased during the 60-d exposure period; whereas concentrations in the sipunculid *Phascolosoma agassizii* appeared to have reached an equilibrium relatively early in the exposure. Experiments on uptake of $^{14}$C-phenanthrene, -chrysene, -dimethylbenz[*a*]anthracene, and -benzo[*a*]pyrene by *M. inquinata* indicated that compounds directly associated with sediment were less available for uptake than those released from sediment to the surrounding seawater. Concentration factors for uptake from sediment were $\leq$0.2, while those for uptake from seawater were 10–1349. Additionally, the heavier molecular weight aromatic compounds tended to be more concentrated in tissue and retained for longer periods of time than the lighter compounds.

*Key words:* petroleum, hydrocarbons, sediments, bivalve, sipunculid, feeding type

ROESIJADI, G., J. W. ANDERSON, AND J. W. BLAYLOCK. 1978. Uptake of hydrocarbons from marine sediments contaminated with Prudhoe Bay crude oil: influence of feeding type of test species and availability of polycyclic aromatic hydrocarbons. J. Fish. Res. Board. Can. 35: 608–614.

Nous avons exposé des animaux benthiques à des sédiments marins contaminés par du pétrole brut de la baie Prudhoe et avons mesuré de façon continue l'absorption des hydrocarbures dans diverses conditions expérimentales. Lorsque l'on compare l'absorption des hydrocarbures aliphatiques et diaromatiques par deux organismes se nourrissant de sédiments, *Macoma inquinata* et *Phascolosoma agassizii* à celle d'un organisme se nourrissant de matière en suspension, *Protothaca staminea*, nous constatons que les premiers accumulent généralement plus d'hydrocarbures que les derniers. D'autres facteurs cependant, telle l'aptitude intrinsèque des espèces à accumuler des hydrocarbures, influent également sur le degré de contamination. Les concentrations d'hydrocarbures chez les espèces bivalves (*M. inquinata* et *P. staminea*) augmentent durant la période d'exposition de 60 jours; les concentrations chez le sipunculide *Phascolosoma agassizii* semblent par ailleurs atteindre un équilibre relativement tôt pendant l'exposition. Des expériences sur l'absorption de $^{14}$C-phénanthrène, -chrysène, -diméthylbenz[*a*]anthracène et -benzo[*a*]pyrène par *M. inquinata* indiquent que les composés directement associés aux sédiments sont moins disponibles pour l'absorption que ceux qui ont été libérés du sédiment dans l'eau de mer ambiante. Les facteurs de concentration applicables à l'absorption à partir des sédiments sont $\leq$0.2, alors que ceux touchant l'absorption à partir de l'eau de mer sont de 10 à 1 349. De plus, les composés aromatiques à poids moléculaires élevés ont tendance à se concentrer davantage dans le tissu et à être retenus plus longtemps que les composés légers.

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It is presently suspected that a significant portion of the petroleum hydrocarbons that enter the marine environment become associated with bottom sediments. Farrington and Quinn (1973) have proposed a process whereby hydrocarbons in seawater become associated with suspended particulate matter, with the resulting aggregates sedimenting to the bottom. Elevated concentrations of hydrocarbons have been reported for marine sediments in the vicinity of several known sources of petroleum contamination (Blumer et al. 1970; Farrington and Quinn 1973; Wharfe 1975; Gilfillan et al. 1976; Krebs and Burns 1977). Although considerable information is available on the toxicity, uptake, and effects of petroleum dissolved or dispersed in seawater (Neff et al. 1976; Anderson 1977; Rice et al. 1977), little is known about interactions between petroleum associated with sediment and benthic infaunal organisms. The latter subject is of interest with respect to the potential consequences of oil inputs to the marine environment, particularly the long-term effects of petroleum contamination.

Of the feeding types described for marine benthic organisms, deposit feeding is one of the more prevalent. Because deposit feeders actively ingest sediment and assimilate associated organic material, it would appear that they would be highly susceptible to contamination from oiled sediment. However, studies to date have indicated little or no uptake of naphthalenes, one of the more toxic components of petroleum (Anderson et al. 1974), by three deposit-feeding species exposed to oil-contaminated sediments (Rossi 1977; Anderson et al. 1977; Roesijadi et al. 1978). We have performed experiments to examine the influence of feeding type on the availability of petroleum hydrocarbons from sediment. The deposit-feeding clam *Macoma inquinata*, sipunculid *Phascolosoma agassizii*, and the suspension-feeding clam *Protothaca staminea* were used as test species. We also examined the uptake of specific $^{14}$C-labeled aromatic hydrocarbons from oil-contaminated sediment by *M. inquinata*.

## Materials and Methods

### Animals

The deposit feeders *Macoma inquinata* and *Phascolosoma agassizii* and the suspension feeder *Protothaca staminea* were collected from the intertidal region of Sequim Bay, Washington. These three species co-habit the same general areas within our collection site. Animals were held in the laboratory in flowing seawater at approximately 10°C and 30‰ for no longer than 1 wk before use in experiments.

### Field Exposure to Prudhoe Bay Crude Oil

Exposure sediment (beach sand: 0.9% ash-free dry weight) was prepared by the method of Anderson et al. (1977) using Prudhoe Bay crude oil and was placed in fiberglass boxes divided into three equal compartments (Anderson et al. 1977), each with bottoms of 1.6-mm$^2$ fiberglass screens. Individuals of *Phascolosoma agassizii*, *M. inquinata*, and *Protothaca staminea* were buried in the exposure sand, with both species of clams in the same compartments. Exposure boxes containing test animals were inserted into the sediment in the intertidal zone of Sequim Bay at approximately the +0.15 m tide level. The fiberglass screen bottoms permitted water exchange in exposure sediment, particularly during fluctuations in tides. Animals in clean sand served as controls. Sediment was sampled prior to installation in the field and at 40 and 60 d of exposure and analyzed for total petroleum hydrocarbons by infrared (IR) spectophotometry (American Petroleum Institute 1958). Animals were sampled at the same times and analyzed for aliphatic and diaromatic hydrocarbons by gas chromatography (Warner 1976). Diaromatic hydrocarbons were identified as to naphthalene, methylnaphthalenes, or dimethylnaphthalenes by comparing retention times with those of standards and with those of the same compounds in Prudhoe Bay crude oil that had been analyzed by gas chromatography–mass spectrometry.

### Laboratory Exposure to Prudhoe Bay Crude Oil and $^{14}$C-Polycyclic Aromatic Hydrocarbons

Short- and long-term laboratory experiments were conducted with *M. inquinata* to examine uptake of specific aromatic hydrocarbons from detritus contaminated with Prudhoe Bay crude oil. Uptake of $^{14}$C-phenanthrene, -chrysene, -dimethylbenz[*a*]anthracene, and -benzo[*a*]pyrene was determined in short-term (7 d) experiments by a previously described technique (Roesijadi et al. 1978). Briefly, detrital material (8.7% ash-free dry weight) that settled out of our flowing seawater system was collected and filtered onto No. 42 Whatman filter paper, then suspended in ~50 mL seawater. Ten microcuries ($\mu$Ci) of the appropriate $^{14}$C-hydrocarbon and 0.033 mL Prudhoe Bay crude oil dissolved together in 1 mL ethyl ether were added to the suspended detritus, mixed thoroughly by shaking, then filtered onto No. 42 Whatman filter paper. The contaminated detritus measured 2000 $\mu$g/g total petroleum hydrocarbons by IR spectrophotometry. Exposures were conducted in all-glass aquaria containing 3 L of 0.45 $\mu$m filtered seawater and the contaminated detritus which formed a thin (1–2 mm) layer on the bottom. Because oil-contaminated sediments can release hydrocarbons to surrounding seawater, one group of test clams was exposed directly to the sediment, while another was suspended in the overlying water column. The latter group was, therefore, exposed to waterborne hydrocarbons released from sediment. Net uptake of both solubilized and sediment-bound hydrocarbons was calculated as described in a previous communication (Roesijadi et al. 1978).

In the long-term experiments, exposures were conducted in three-compartmented sediment trays similar to those described above. Each sediment tray was filled with 3 kg Sequim Bay sand per compartment, then placed in a holding tank with flowing seawater and a simulated daily tidal cycle. Sediment trays were placed at a level such that "high tide" (maintained by an overflow standpipe in the holding tank) did not overflow the upper edges of the trays. At "low tide," seawater drained from the trays through the fiberglass screen bottoms of each compartment. The principal water exchange in the exposure trays occurred through the tray bottoms. The flushing prevented formation of anoxic sediment, a problem encountered in earlier experiments. Test clams were then placed in the clean sand of each tray compartment.

TABLE 1. Aliphatic and diaromatic hydrocarbon concentrations (μg/g wet weight) in *Phascolosoma agassizii*, *Macoma inquinata*, and *Protothaca staminea* exposed to oil-contaminated sediment. Samples[a] were obtained after 40- and 60-d exposures.

| Treatment/species | $C_{12}$–$C_{28}$ | Naphthalene | Methyl-naphthalenes | Dimethyl-naphthalenes | Total diaromatics[b] |
|---|---|---|---|---|---|
| Controls | | | | | |
| *P. agassizii* | <0.10 | <0.005 | <0.005 | <0.01 | <0.02 |
| *M. inquinata* | <0.10 | <0.005 | <0.005 | <0.01 | <0.02 |
| *P. staminea* | <0.10 | <0.005 | <0.005 | <0.01 | <0.02 |
| 40-d exposure | | | | | |
| *P. agassizii* | 1.90 | <0.005 | 0.23 | 0.60 | 0.83 |
| *P. agassizii* | 0.73 | <0.005 | 0.01 | 0.15 | 0.16 |
| *M. inquinata* | 0.69 | <0.005 | 0.06 | 0.89 | 0.96 |
| *P. staminea* | <0.10 | <0.005 | <0.005 | <0.01 | <0.02 |
| 60-d exposure | | | | | |
| *P. agassizii* | 1.48 | 0.01 | 0.06 | 0.18 | 0.25 |
| *M. inquinata* | 0.54 | 0.02 | 0.27 | 2.39 | 2.68 |
| *M. inquinata* | 3.62 | 0.02 | 0.26 | 1.96 | 2.24 |
| *P. staminea* | 0.10 | <0.005 | 0.02 | 0.16 | 0.18 |

[a]Each sample consisted of two to four pooled individuals; clams were shucked prior to extraction.
[b]Total diaromatics include naphthalene, methylnaphthalenes, and dimethylnaphthalenes only.

At "high tide," 25–30 g of detritus containing 2000 μg/g total petroleum hydrocarbons and 1 μCi/g $^{14}$C-phenanthrene, -chrysene, or -benzo[*a*]pyrene (prepared as described above) was suspended in ~100 mL seawater and added to each compartment. The detritus settled on the surface of the sand, forming a layer about 1–2 mm thick. Clams and sediment cores were sampled at intervals up to 60 d of exposure. The sediment cores were extracted and analyzed for total petroleum hydrocarbons and radioactivity. Clams were transferred to clean seawater for 24 h to allow purging of gut contents, then assayed for radioactivity.

In short- and long-term experiments, extraction of $^{14}$C-hydrocarbons from sediment and clam tissue followed the method of Warner (1976) with the minor modifications described by Roesijadi et al. (1978). Diethyl ether extracts of saponified samples were counted in Aquasol II (New England Nuclear, Boston, Mass.) using a Beckman LS-150 liquid scintillation spectrometer. Total petroleum hydrocarbons in sediment were analyzed as described above. All solvents were distilled in glass (Burdick and Jackson, Muskegon, Mich.). $^{14}$C-phenanthrene (11.3 mCi/mM), -chrysene (6.3 mCi/mM), and -benzo[*a*]pyrene (51 mCi/mM) were purchased from Amersham/Searle Corporation (Arlington Heights, Ill.). $^{14}$C-dimethylbenz[*a*]anthracene (10.55 mCi/mM) was purchased from New England Nuclear (Boston, Mass.).

## Results

### Field Exposure to Prudhoe Bay Crude Oil

Concentrations of total petroleum hydrocarbons in exposure sediment were 887.4 μg/g initially, then declined to 443.8 and 420.6 μg/g after 40–60 d in the field, respectively. Decreases in sediment hydrocarbon concentrations can probably be attributed primarily to release to the surrounding seawater, with uptake by animals and microbial photooxidation as minor factors.

Accumulation of hydrocarbons by animals was generally higher in the two deposit-feeding species, *Phascolosoma agassizii* and *M. inquinata*, than in *Protothaca staminea*, the suspension-feeding clam (Table 1). After the 40-d exposure, levels in *P. staminea* were below our detection limits, while those in *Phascolosoma agassizii* and *M. inquinata* ranged from 0.69 to 1.90 μg/g $C_{12}$–$C_{28}$ aliphatics and 0.16 to 0.89 μg/g total diaromatics. The alkylated forms, primarily the dimethylnaphthalenes, were dominant in the latter group. At 60 d, concentrations in *P. agassizii* were similar to those observed at 40 d, while those in *M. inquinata* and *Protothaca staminea* were higher than 40-d levels. Large increases in the concentrations of dimethylnaphthalenes could account for most of the increase in *M. inquinata*. In *P. staminea*, detectable levels were observed at 60 d with all compounds except naphthalene. Concentrations of 0.10 μg/g aliphatics and 0.18 μg/g total diaromatics were present in these clams.

### Laboratory Exposure to Prudhoe Bay Crude Oil Containing $^{14}$C-Polycyclic Aromatic Hydrocarbons

Clams (*M. inquinata*) were exposed in a system containing detritus initially contaminated with 2000 μg/g of Prudhoe Bay crude oil and 10 μCi of $^{14}$C-phenanthrene, -chrysene, dimethylbenz[*a*]anthracene, or -benzo[*a*]pyrene for 1 wk. Tissue, sediment, and seawater concentrations of $^{14}$C-radioactivity at 7 d, as well as concentration ratios for uptake from sediment and seawater, are presented in Table 2. Equivalent concentrations in micrograms per gram or micrograms per millilitre (calculated from specific activity) are also presented for each hydrocarbon. We recognize that our measurements of $^{14}$C-radioactivity may include metabolites as well as the parent compound. We did not

TABLE 2. Uptake of $^{14}$C-aromatic hydrocarbons from sediment by *Macoma inquinata* in short-term exposures. Clams were exposed for 7 d to sediment containing initial levels of 2000 $\mu$g/g Prudhoe Bay crude oil spiked with 10 $\mu$Ci of the hydrocarbon indicated.

| Parameter | Phenanthrene | Chrysene | Dimethyl-benz[*a*]anthracene | Benzo[*a*]pyrene |
|---|---|---|---|---|
| Net uptake from sediment dpm/g ($\mu$g/g) | 13 382 (0.096) | 18 917 (0.308) | 27 846 (0.297) | 26 082 (0.059) |
| Uptake from seawater dpm/g ($\mu$g/g) | 5 297 (0.038) | 18 310 (0.297) | 79 160 (0.856) | 16 451 (0.037) |
| Concn in sediment at 7 d dpm/g ($\mu$g/g) | 68 285 (0.49) | 516 670 (8.37) | 428 749 (4.63) | 255 617 (0.64) |
| Concn in seawater at 7 d dpm/mL ($\mu$g/mL) | 517 ($3.7\times10^{-3}$) | 26 ($4.3\times10^{-4}$) | 59 ($6.3\times10^{-4}$) | 19 ($4.3\times10^{-5}$) |
| Concn factor for uptake from sediment[a] | 0.20 | 0.04 | 0.06 | 0.09 |
| Concn factor for uptake from seawater[b] | 10.3 | 694 | 1349 | 861 |

[a]Calculated as net uptake from sediment divided by concentration in sediment at 7 d.
[b]Calculated as net uptake from seawater divided by concentration in seawater at 7 d.

TABLE 3. Concentrations ($\bar{x} \pm 1$ SE dpm/g; $\bar{x}$ $\mu$g/g) of $^{14}$C-aromatic hydrocarbons in sediment during long-term exposure of *Macoma inquinata* to contaminated sediment.

| Parameter | Phenanthrene | Chrysene | Benzo[*a*]pyrene |
|---|---|---|---|
| Initial detritus[a] dpm/g ($\mu$g/g) | 1 921 393 $\pm$ 4 595 (13.8) | 1 680 838 $\pm$ 41 164 (27.3) | 1 819 491 $\pm$ 72 605 (3.79) |
| Initial detritus + sand[b] dpm/g ($\mu$g/g) | 18 924 $\pm$ 975 (0.136) | 16 641 $\pm$ 408 (0.271) | 12 611 $\pm$ 542 (0.025) |
| Final sediment[c] dpm/g ($\mu$g/g) | 103 $\pm$ 8 (0.001) | 868 $\pm$ 113 (0.014) | 2 521 $\pm$ 482 (0.006) |
| Geometric mean concn in sediment during exposure[d] dpm/g ($\mu$g/g) | 543 (0.004) | 3 271 (0.053) | 2 948 (0.007) |

[a]Samples of contaminated detritus prior to addition to exposure system.
[b]Calculated from the concentration in initial detritus and combined weights of detritus (25 or 30 g) and sand (3000 g) in an exposure compartment.
[c]Direct analysis of core samples at 56 d for phenanthrene, 52 d for chrysene, and 42 d for benzo[*a*]pyrene.
[d]Calculated as geometric means of samples taken at all sampling times; levels of hydrocarbons in initial detritus plus sand above were used as initial samples.

feel the need to discriminate completely between hydrocarbon and metabolite in this phase of our study.

Since equivalent levels of radioactivity, not equivalent levels of aromatic hydrocarbons, were added to the exposure detritus; concentrations in tissue for the different $^{14}$C-hydrocarbons cannot be compared directly. However, for individual compounds, uptake from sediment can be compared with uptake from the seawater. Concentrations in tissue for both sediment and seawater routes of uptake were within 1 order of magnitude for a single compound, indicating that the contribution from either source was similar. However, because concentrations in sediment were considerably higher than those in the seawater, availability of hydrocarbons from sediment was, therefore, much lower than from the seawater. Concentration ratios support this hypothesis. Tissue–sediment hydrocarbon concentration ratios were $\leq$0.2 for all four compounds, indicating no biological magnification in 7 d, whereas tissue–seawater concentration ratios were high ($\gg$1) and indicative of biological magnification. Furthermore, the latter values exhibited a strong correlation with the molecular weights of the hydrocarbon; concentration ratios increased from 10.3 to 861 as the compounds increased in molecular weight from phenanthrene to benzo[*a*]pyrene. Dimethylbenz[*a*]anthracene, a four-ringed aromatic with two methyl side-chains, was anomalous in that its concentration factor for uptake from seawater exceeded that of benzo[*a*]pyrene.

In long-term exposures of *M. inquinata* to $^{14}$C-phenanthrene, -chrysene, or -benzo[*a*]pyrene, concentrations of the hydrocarbons in sediment decreased with time (Table 3). During the course of exposure, the detritus, which had initially settled onto the surface of the sand in each exposure compartment, penetrated into the interstitial spaces of the sand as a result of the simulated tidal fluxes. Initial hydrocarbon concentrations for detritus were corrected to account for the total sediment load (= sand + detritus); these values could then be compared directly with those of core samples taken at later sampling periods and used for calculation of geometric mean levels during the exposure. At the beginning of the exposure, clams were seen to be actively siphoning contaminated detritus from the sedi-

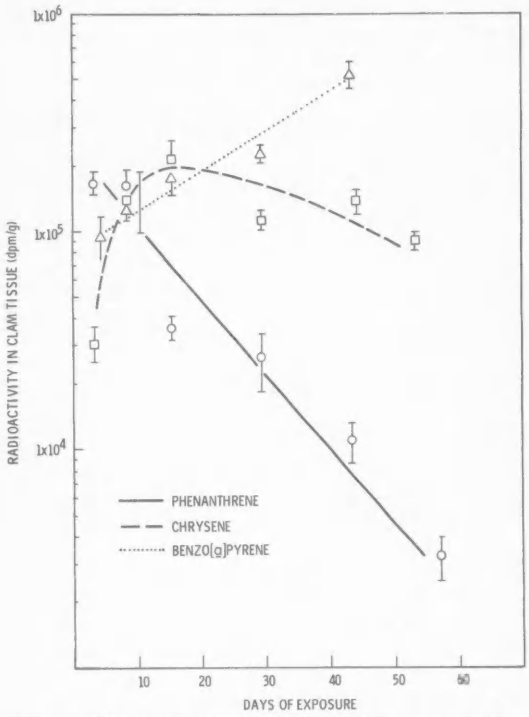


FIG. 1. $^{14}$C-radioactivity in tissue of *Macoma inquinata* during long-term exposure to contaminated sediment. Means ± 1 SE are shown for $^{14}$C-phenanthrene, -chrysene, and -benzo[*a*]pyrene.

ment surface. This behavior undoubtedly resulted in the initially high concentrations of hydrocarbons in tissue observed with all three compounds (Fig. 1). With subsequent exposure, the pattern of uptake by the clams was unique for each of the compounds. Following the initial uptake described above, $^{14}$C-phenanthrene concentrations decreased exponentially during the exposure period, while benzo[*a*]pyrene levels increased with time. Chrysene, which is intermediate in molecular weight to phenanthrene and benzo[*a*]pyrene, increased with time up to day 14, then decreased with subsequent exposure — a pattern intermediate to that of the other two compounds. The differences in the curves for the three hydrocarbons were consistent with a relationship between uptake–retention and molecular structure. In this regard, then, the results of the long-term exposures agreed with those of short-term experiments described previously.

## Discussion

The results of our field and laboratory experiments were consistent with previous findings that naphthalenes in oil-contaminated sediments are not readily accumulated by benthic detritivores (Rossi 1977; Anderson et al. 1977; Roesijadi et al. 1978) and we extended the data to include aliphatic and higher molecular weight aromatic compounds.

In the field experiment described in this report, uptake of aliphatic and diaromatic petroleum hydrocarbons was examined in three benthic animals exposed to sediment contaminated with Prudhoe Bay crude oil. The concentrations of hydrocarbons in sediment (887 μg/g initially and 420 μg/g after 60 d) were extremely high, but consistent with levels reported for heavily contaminated areas (Gilfillan et al. 1976; Krebs and Burns 1977). In contrast, tissue hydrocarbon levels reported here after 40–60 d of exposure were relatively low. Maximum levels observed in tissue did not exceed 6 μg/g combined aliphatic and diaromatic hydrocarbons. The concentration of $C_{12}$–$C_{28}$ *n*-alkanes in tissue was roughly equivalent to those of the diaromatic hydrocarbons, with the dimethylnaphthalenes predominant in the latter fraction. Feeding type of test species may have influenced the bioavailability of hydrocarbons. The deposit feeders accumulated hydrocarbons to a greater extent than the suspension feeder; this pattern was evident at 40 d. At 60 d, however, the data seemed to indicate a difference in uptake patterns between the sipunculids and the bivalve species and suggested intrinsic differences in abilities of different species to accumulate hydrocarbons. Hydrocarbon concentrations in the sipunculid *Phascolosoma agassizii* at this time were not different from 40-d levels; whereas those in the bivalve *M. inquinata* and *Protothaca staminea* represented measurable increases. Dilution of leached hydrocarbons by overlying seawater can probably explain the low levels observed in *P. staminea*, since suspension feeders would not ordinarily be exposed directly to contaminated sediments or interstitial water. When compared with other organisms, bivalves have been shown to take up hydrocarbons from seawater at a slow rate for a prolonged period of time until equilibrium is reached (Stegeman and Teal 1973; Neff et al. 1976). Tissue hydrocarbon concentrations as high as several hundred micrograms per gram were reported for oysters and clams. Thus, neither *M. inquinata* nor *P. staminea* apparently approached their maximum capacity to accumulate hydrocarbons during our study. With the sipunculid *Phascolosoma agassizii*, however, tissue levels of naphthalenes were stable after the first 40 h of a 2-wk exposure to oil-contaminated sediment (Anderson et al. 1977). These results were consistent with our observations in longer exposures.

When exposed to detritus containing radiolabeled polycyclic aromatic hydrocarbons spiked into Prudhoe Bay crude oil, accumulation of these compounds by *M. inquinata* was also low. We found that compounds directly associated with particulate matter were less available for uptake than that fraction released to the surrounding seawater. As a result of our findings, we suspect that hydrocarbons present in interstitial water may be a prime source of contamination for infaunal species and probably accounted for much of the uptake

by *P. agassizii* and *M. inquinata* in the field experiment described above. Concentration factors for 7-d exposures (based on uptake from seawater) correlated positively with the increasing molecular weights of the aromatic hydrocarbons examined here. Such a relationship was not unexpected and was predictable from our current understanding of bioconcentration of organic compounds and partition coefficients (Neely et al. 1974). Dimethylbenz[*a*]anthracene was an exception to the above trend and may have represented the influence of aliphatic side chains on the aromatic rings. During continuous exposure to aromatic hydrocarbons, concentrations in tissue would be expected to reach an equilibrium with external concentrations, with lighter molecular weight hydrocarbons tending to approach equilibrium values at a faster rate than heavier compounds. Actual equilibrium concentrations in tissue would depend on the concentration in the external medium. Our longer term tests indicated that phenanthrene concentrations reached equilibrium, presumably with molecules leached from sediment to seawater, within 2 d and chrysene within 14 d. Subsequent declines in tissue-hydrocarbon concentrations reflected new equilibrium concentrations in response to declining levels of sediment. Benzo[*a*]pyrene, however, appeared not to have reached an equilibrium during our experiment, since levels in tissue increased throughout the exposure period despite the declining levels in sediment.

At the present time, there is considerable interest in the polycyclic aromatic hydrocarbons from the standpoint of their suspected carcinogenic properties (Christensen et al. 1975). Although concentrations of polycyclic aromatic hydrocarbons in crude oils are very low (Pancirov and Brown 1975), chronic inputs of petroleum into the marine environment can result in significant levels of the compounds in sediment (McLeod et al., 1977). Our data have indicated that higher molecular weight compounds such as benzo[*a*]pyrene will be relatively persistent in tissue of exposed bivalves. Benzo[*a*]pyrene is already being monitored in marine bivalves as an indicator of environmental contamination (Dunn and Stich 1976; Dunn and Young 1976; Mix et al. 1977), and other polycyclic aromatic hydrocarbons have been identified as "potentially useful indicators of petroleum contamination" (McLeod et al. 1977). Analysis for such compounds in the tissue of benthic organisms, particularly deposit-feeding bivalves, may prove to be a sensitive measure of petroleum contamination of the environment.

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# Aromatic Hydrocarbons in Benthic Organisms from Coastal Areas Polluted by Iranian Crude Oil[1]

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GRAHL-NIELSEN, O., J. T. STAVELAND, AND S. WILHELMSEN. 1978. Aromatic hydrocarbons in benthic organisms from coastal areas polluted by Iranian crude oil. J. Fish. Res. Board Can. 35: 615–623.

This investigation was begun 1 mo after a spill of 2000 tons of Iranian crude oil polluted shores along the west coast of Norway in February 1976. It is based on mass-fragmentographic analysis of naphthalene, methyl-, dimethyl-, and trimethylnaphthalenes, phenanthrene, methyl- and dimethylphenanthrenes, dibenzothiophene, methyl-, dimethyl-, and trimethyldibenzothiophenes. Samples of stranded oil, water, and seven species of benthic organisms were collected periodically during the following year. During the 1st mo of weathering the total amount of these aromatic compounds had decreased from 1.22% in the original oil to 0.76%, with a further decrease to 0.2–0.3% during the next 3-4 mo. In the water the concentration of these compounds was approximately 2 μg/L (ppb) at the start of the sampling. At one of the investigated locations a chemical dispersant was used in cleaning the shores. This resulted in higher concentrations of the aromatic compounds in the water, up to 12 μg/L. The organisms studied had accumulated aromatic hydrocarbons up to an excess of 20 μg/g wet weight, 1 mo after the spill. Although seasonal and individual differences were large, the dibenzothiophenes appeared to be retained to a larger extent relative to the naphthalenes and phenanthrenes.

*Key words:* oil spill, benthic organisms, naphthalenes, phenanthrenes, dibenzothiophenes, mass fragmentography, petroleum

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Cette recherche a commencé 1 mo après qu'un déversement accidentel de 2 000 tonnes de pétrole brut iranien ait pollué les rives de la côte occidentale de la Norvège en février 1976. Elle est fondée sur une analyse par fragmentographie de masse du naphtalène, des méthyl-, diméthyl- et triméthylnaphtalènes, phénanthrène, méthyl- et diméthylphénanthrènes, dibenzothiophène, méthyl-, diméthyl- et triméthyldibenzothiophènes. Au cours de l'année qui suivit le déversement, nous avons prélevé périodiquement des échantillons de pétrole échoué, d'eau et de sept espèces d'organismes benthiques. Durant le premier mois d'altération à l'air, la quantité totale de ces composés aromatiques passa de 1.22% dans le pétrole originel à 0.76%, et diminua encore à 0.2–0.3% durant les 3–4 mo suivants. La teneur des ces composés dans l'eau était approximativement 2 μg/L (ppb) au début de l'échantillonnage. A un des endroits sous étude, on a utilisé un dispersant chimique pour nettoyer les rives. Cela eut pour effet d'augmenter les concentrations de composés aromatiques dans l'eau, jusqu'à 12 μg/L. Les organismes étudiés accumulèrent des hydrocarbures aromatiques, jusqu'à excès de 20 μg/g de poids humide, 1 mo après le déversement. En dépit de fortes différences saisonnières et individuelles, les dibenzothiophènes semblent être retenus à un plus haut degré comparativement aux naphtalènes et phénanthrènes.

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WE describe in this paper the determination of aromatic hydrocarbons in the following species of benthic animals: bread-crumb sponge, *Halichondria panicea*; dahlia anemone, *Tealia felina*; the starfish *Asterias rubens*; the mussel *Mytilus edulis*; the limpet *Patella vulgata*; the periwinkle *Littorina littorea*; and shore crab, *Cancer pagurus*. Samples of these species were collected from two polluted bays and one unpolluted reference site (Fig. 1) during a 1-yr period following the spill of approximately 2000 tons of Iranian crude oil at the approach to a refinery near Stavanger. The oil drifted on relatively calm water northward, where part of it reached the southern shoreline of Karmøy after 2 d.

We decided to investigate the content of aromatic hydrocarbons in organisms exposed to the oil, mainly

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[3]Submitted in partial fulfillment of the requirements for the degree of Cand. Real.

[4]Responsible for the chemical analysis.

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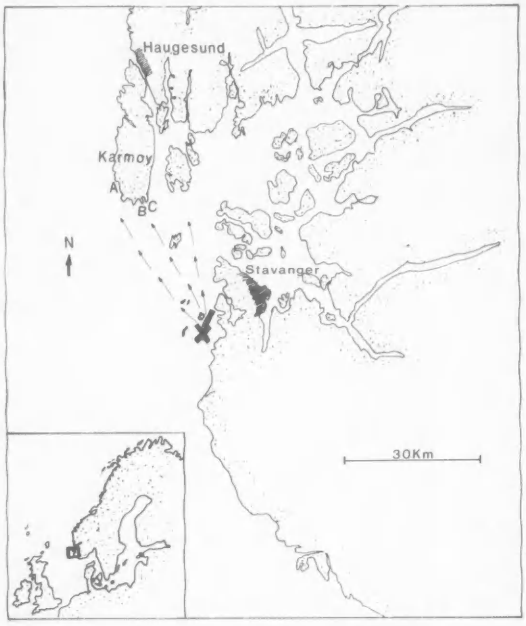



FIG. 1. Area in southwestern Norway affected by the oil-spill. T.T. *Drupa* touched the shoals at X and the oil was spilled in the crosshatched area. Arrows indicate the drift of the oil during the first two days. The three sampling locations were near A, B, and C; chemical dispersant was used at B, and C was the unpolluted reference location.

because these components are thought to be the most toxic in mineral oil (Rice et al. 1977) and are also among the most water soluble (Shaw 1977) and hence more available to marine organisms. Of the numerous aromatic hydrocarbons present in crude oil the interest was focused on naphthalenes, phenanthrenes, and dibenzothiophenes.

The naphthalenes were investigated because they appear to be among those petroleum hydrocarbons that are accumulated most rapidly and retained the longest by marine fish and invertebrates (Rossi and Anderson 1977). Phenanthrenes were included because toxicity of aromatic hydrocarbons is thought to increase with increasing number of fused rings (Rice et al. 1977).

The aromatic thiophenes are rarely encountered in investigations of oil pollution. They appear in significant amounts in crude oil. Landner and Hagstrøm (1975) suggested that these compounds may be preferentially concentrated in the marine environment. The dibenzothiophenes are well suited for mass-fragmentographic analysis. Consequently, there were several reasons for their inclusion in the present study.

## Materials and Methods

Oil, water, and organisms were sampled on March 12, 15, 19, 25, April 10, 22, 26, May 4, 14, June 6, July 9, August 27, September 28, November 22, February (1977) 29, and March 15, and were taken as nearly as possible from the same locations. Everything was left in its natural condition at the sampling sites.

The oil was sampled with small glass vials with Teflon-lined caps and was kept refrigerated until analysis. Water was sampled in 2.8-L bottles from approximately 30 cm below the surface. Care was taken to avoid contamination by the surface film. Thirty millilitres of column-distilled dichloromethane was added to the bottles to prevent microbial activity. The organisms were collected by hand or, if necessary, by scuba diving. Direct contact with oil was avoided, and samples were wrapped in clean aluminium foil and frozen.

Preparation of the samples for the final analysis was performed as follows: Oil samples were dissolved in an appropriate volume of dichloromethane; precleaned sodium sulfate was added to dry the solution. A subsample was transferred to a clean vial and the dichloromethane evaporated under a stream of dry nitrogen gas. The oily residue was dissolved in carbon disulfide, and a known amount of anthracene was added as internal standard.

For extraction of the water samples, water and dichloromethane were transferred from the sample bottle to a 3-L separatory funnel with Teflon stopcock and stopper. The sample bottle was rinsed with another 25 mL dichloromethane which was thereafter transferred to the separatory funnel. The extraction was performed by thorough shaking for 1 min, followed by separation of the dichloromethane. This extraction was repeated twice with 25 mL each time. The extract was dried with approximately 5 g of precleaned sodium sulfate. Two separate aliquots of the extract were withdrawn, one for analysis of total hydrocarbons and one for analysis of the aromatic hydrocarbons. The former was concentrated on a rotary evaporator at 15–20°C under reduced pressure from a water aspirator, and the residue was quantitatively transferred to a capsule of a Perkin-Elmer automatic sampler. To the latter, known amounts of fluorene and anthracene were added as internal standards. The solvent was carefully removed and the residue dissolved in approximately 15 μL carbon disulfide.

After thawing the animal samples, we removed shells where necessary. Samples were blotted on filter paper and the wet weight of the soft tissue determined. The average sample size was 5–10 g. Known amounts of fluorene and anthracene were added as internal standards and then the saponification method of Farrington and Medeiros (1975) was used for freeing tissue hydrocarbons. After the pentane extract was cleaned on a short $SiO_2$-column, the solvent was carefully evaporated and the residue dissolved in approximately 20 μL carbon disulfide.

The final analysis was performed with gas chromatography in two different ways:

For the determination of total hydrocarbons in the water the samples in the capsules of the Perkin-Elmer automatic sampler were chromatographed on a SP-2100-packed glass column in a Perkin-Elmer 900 gas chromatograph with nitrogen, 20 mL/min, as carrier gas and flame ionization detector. The total area of the chromatograms was determined by planimetry and converted to concentration units by comparison with chromatograms of Iranian crude reference oil.

For the analysis of the selected aromatics a Finnigan 9500 gas chromatograph was used. Approximately 0.1 μL of the carbon disulfide solution was chromatographed on a 20-m SE-54 glass capillary column from Jaeggi, CH-

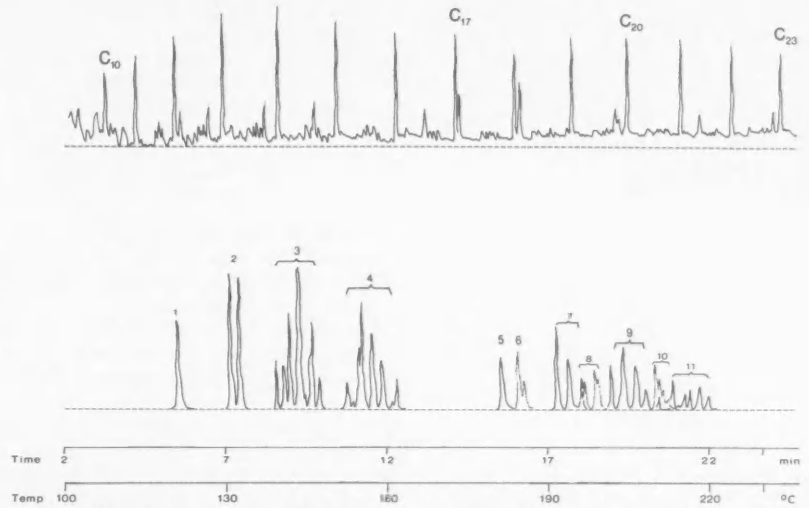



FIG. 2. Gas chromatogram of Iranian crude oil on capillary column based on total ion current (TIC) detection (*upper trace*). Mass chromatogram of the aromatics reconstructed from the TIC-chromatogram and enlarged ~40 times (*lower trace*). Peaks represent the following: 1 — naphthalene, 2 — methylnaphthalenes, 3 — dimethylnaphthalenes, 4 — trimethylnaphthalenes, 5 — dibenzothiophene, 6 — phenanthrene (dotted line), 7 — methyldibenzothiophenes, 8 — methylphenanthrenes (dotted line), 9 — dimethyldibenzothiophenes, 10 — dimethylphenanthrenes (dotted line), 11 — trimethyldibenzothiophenes.

9043 Trogen, Switzerland. Helium served as carrier gas at 2 mL/min, and the column was connected by a 30-cm platinum capillary without separator to the ion chamber of a Finnigan 3200 mass spectrometer. By selected-ion monitoring (mass fragmentography) the following molecular ions were recorded: 128 — naphthalene, 141 — methylnaphthalene minus one mass unit and dimethylnaphthalene minus 15 mass units, 166 — fluorene, 170 — trimethylnaphthalene, 178 — phenanthrene and anthracene, 184 — dibenzothiophene, 192 — methylphenanthrene, 198 — methyldibenzothiophene, 206 — dimethylphenanthrene, 212 — dimethyldibenzothiophene, and 226 — trimethyldibenzothiophene. Data were stored for further treatment in a Finnigan 6100 computer. The various peaks were integrated after subtraction of the background signal. The areas of the peaks were converted to concentration units by comparison with the internal standards, taking the relative response of each compound into consideration.

The precision of the mass fragmentographic analysis was evaluated by repeated analysis of a sample of the weathered oil. For the determination of the total amount of aromatics the standard deviation was ±2%, while the standard deviation for the single components varied between ±2 and ±7%.

By spiking samples of mussels from uncontaminated water with known amounts of a solution of the weathered oil the recovery of the saponification–extraction procedure was found to be 78.9 ± 3.9%. However, the loss of about 20% during the analytical procedure was only of theoretical interest in this case because the quantification was based on the internal standards, which were added before the workup started. By using these standards, 105 ± 2% of the added aromatics was recovered. The reproducibility for single components was between 2 and 7%, as was also the case for the direct analysis of the samples of weathered oil. This estimate of the recovery of the aromatics from the spiked tissue is not necessarily valid for the recovery of tissue-bound aromatics in contaminated animals.

## Results and Discussion

A gas chromatogram of Iranian crude oil is shown in Fig. 2, together with a mass fragmentogram of the aromatics in question based on selected-ion monitoring. The mass fragmentogram is enlarged, but the various peaks are located with correct retention times relative to the gas chromatogram above. The aromatics accounted for 1.22% by weight of the crude oil. The naphthalenes constituted 71.2% of this, the phenanthrenes, 6.6%, and the dibenzothiophenes, 21.8%. In oil sampled March 19, i.e. weathered for 1 mo, the relative amount of aromatics had fallen to 0.76%, and in samples collected at later stages their amount had decreased further to 0.2–0.3%.

With respect to the whole oil it had lost about 50% of its light ends during the first 2 wk after the spill (Grahl-Nielsen et al. 1976). This disappearance must be mainly due to evaporation and dissolution, and involve components up to about the $C_{17}$ normal alkane. As seen from Fig. 2, naphthalene and its alkyl derivatives fall in this range. The disappearance of the naphthalenes was therefore relatively more rapid than that of the phenanthrenes. The dibenzothiophenes also decreased drastically during the 1st mo. Later dimethylnaphthalene also disappeared.

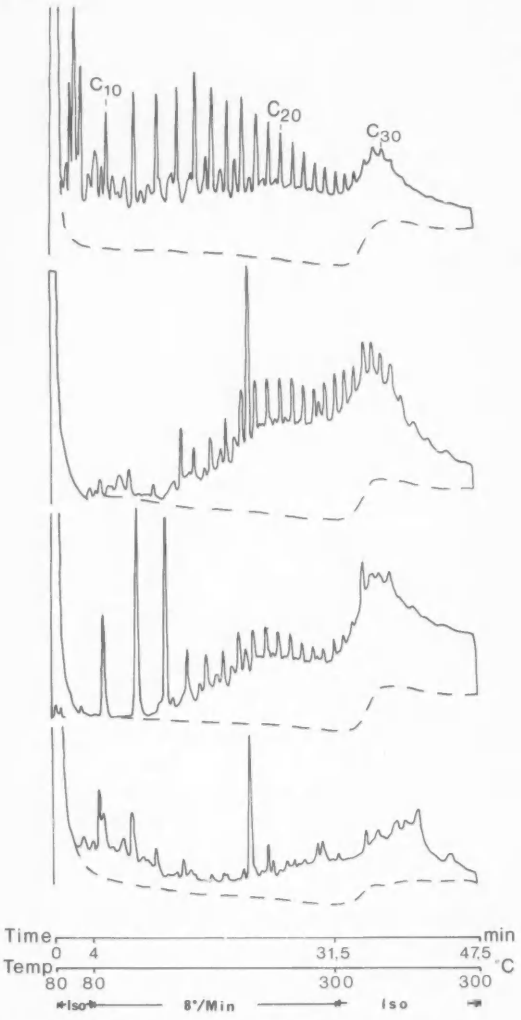



FIG. 3. Gas chromatograms on a packed column of (*from top to bottom*) Iranian crude oil, extracts of water from location A, location B (where a chemical dispersant was used), and the reference location C.

The phenanthrenes and dibenzothiophenes have volatilities equivalent to the range between $C_{17}$ and $C_{22}$ (Fig. 2). Evaporation of these compounds from the oil was therefore negligible. However, contrary to a doubling of their share of the total oil, which would have been the case if they had not been weathered, they decreased to less than half of their original amount. This means that they were washed out of the remaining oil because of their higher water solubility relative to the other components in the remaining oil. There were, however, notable differences from sample to sample due to differences in appearance and exposure of the oil.

TABLE 1. Total amount of naphthalenes, phenanthrenes, and dibenzothiophenes in water ($\mu$g/L) collected monthly at two locations during the first half year of the investigation.

| Location | Mar. | Apr. | May | June | July | Aug. |
|---|---|---|---|---|---|---|
| A | 2.0 | 0.4 | 0.1 | 0.3 | 1.2 | 0.6 |
| B | 12.1 | 1.6 | 3.3 | — | 0.5 | 0.2 |

The analytical procedure with gas chromatography on a packed column with flame ionization detector and integration of the total area of the resulting chromatogram gave only a rough indication of the amounts of hydrocarbons present in the water. The main reason for this was the relatively large amount of organic components of natural origin that was extracted together with the hydrocarbons. This was reflected in the analysis of the water samples from the reference site (Fig. 3). Assuming that these organics gave the same average detector response as the Iranian crude oil, their concentration was up to an excess of 50 $\mu$g/L. There was a large variation in these values even in parallel samples, probably because of patchiness. Accordingly, only those chromatograms with total integrals equivalent to $>100$ $\mu$g/L could be taken into consideration. Two other requirements had to be fulfilled by the chromatograms for use in the determination of the total hydrocarbons in the water. They had to show a characteristic alkane pattern, and the maximum of the unresolved complex mixture had to appear between $C_{17}$ and $C_{19}$.

The following results were obtained. Water collected on March 12 and 27 on the location where dispersants had not been used contained significant amounts of hydrocarbons in the range 200–300 $\mu$g/L. The similarity between the chromatograms of the extracts of the water and the chromatogram of the reference oil (Fig. 3) suggests that most of the oil in the water appeared as an oil-in-water emulsion. By April 9 the amount in the water had decreased to $<100$ $\mu$g/L, and from April 24 onwards it was not possible to detect oil in the water by this method.

On the location where chemical dispersant was used the amounts of oil in the water were considerably

FIG. 4. Total amounts ($\mu$g/g wet weight) of the aromatics naphthalenes, phenanthrenes, and dibenzothiophenes in the various animals. Circles represent data from location A and squares from location B. Heavy arrow on the abscissa indicates the time of the spill and the three light arrows indicate the times of application of chemical dispersant on location B. The left-hand graph of *C. pagurus* represents muscle tissue; the right, hepatopancreas. Note the larger scale on the ordinate of the crab graphs.

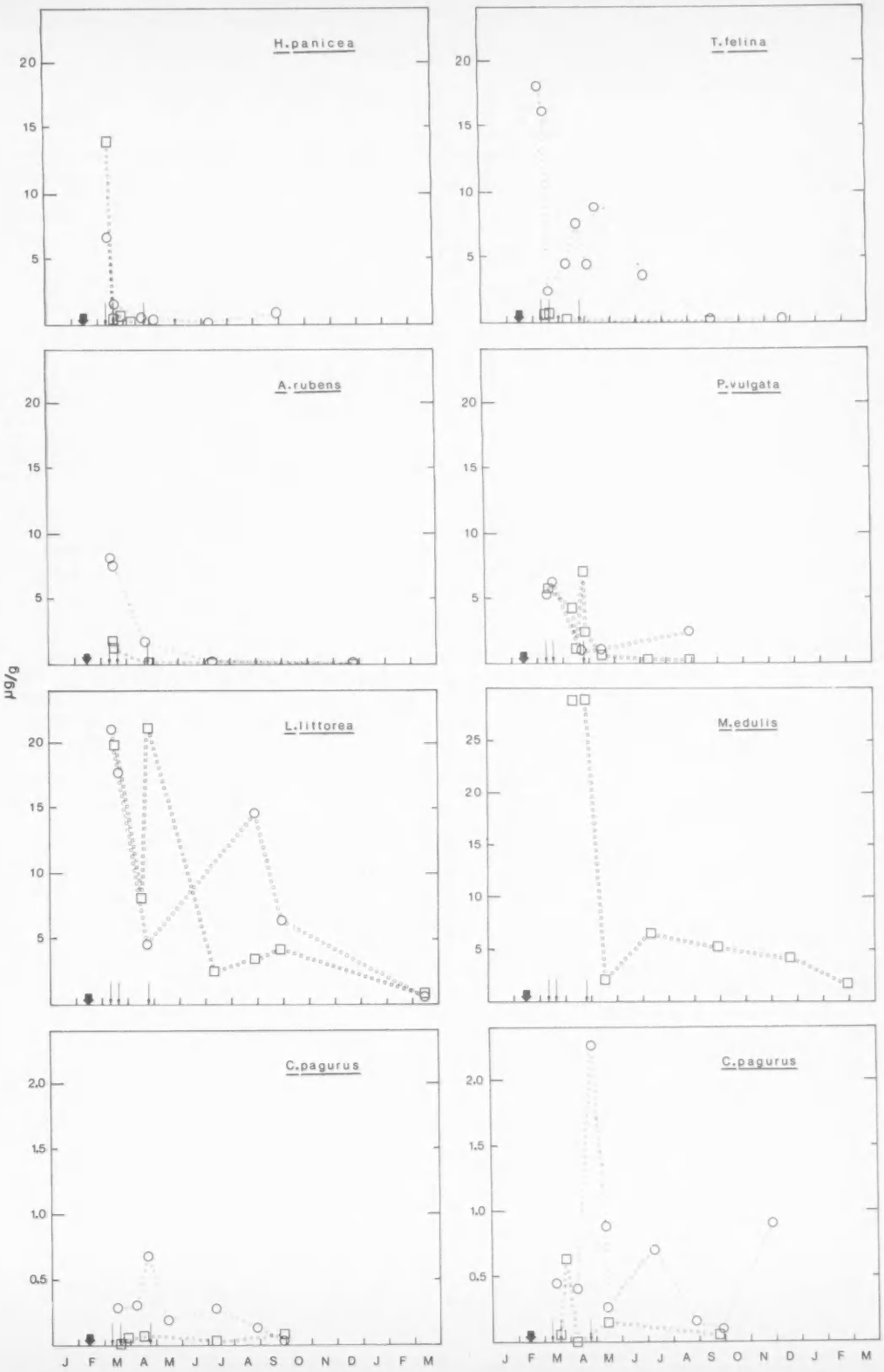

H.panicea
T.felina
A.rubens
P.vulgata
L.littorea
M.edulis
C.pagurus
C.pagurus
µg/g
J F M A M J J A S O N D J F M

TABLE 2. Relative amounts of the various alkylated naphthalenes, phenanthrenes, and dibenzothiophenes in Iranian crude oil, weathered oil, and tissue of animals sampled in the beginning of the investigation. N — naphthalene, MN — methylnaphthalenes, DMN — dimethylnaphthalenes, TMN — trimethylnaphthalenes, P — phenanthrene, MP — methylphenanthrenes, DMP — dimethylphenanthrenes, D — dibenzothiophene, MD — methyldibenzothiophenes, DMD — dimethyldibenzothiophenes, TMD — trimethyldibenzothiophenes.

| Location | | Weathered oil | Sponge | | Anemone | | Starfish | | Limpet | | Winkle | | Mussel |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Iranian crude | A Mar. 19 | A Mar. 12 | B Mar. 14 | A Mar. 19 | B Mar. 19 | A Mar. 15 | B Mar. 14 | A Mar. 19 | B Mar. 14 | A Mar. 19 | B Mar. 15 | B Apr. 10 |
| Total aromatics (μg/g) | | | 6.7 | 13.7 | 18.0 | 0.6 | 7.0 | 1.7 | 6.2 | 5.4 | 17.7 | 19.8 | 28.7 |
| % N | 6.5 | 0.1 | 0.2 | — | 1.1 | 3.7 | 0.8 | 6.1 | 0.7 | 0.6 | 0.0 | — | 0.2 |
| % MN | 24.2 | 3.0 | 3.0 | — | 0.5 | 2.6 | 1.0 | 4.7 | 3.0 | 8.0 | 2.3 | 6.1 | 0.3 |
| % DMN | 31.2 | 28.2 | 10.6 | 14.6 | 11.2 | 12.6 | 15.4 | 12.3 | 35.4 | 32.1 | 15.6 | 32.4 | 4.1 |
| % TMN | 9.5 | 21.5 | 6.4 | 16.8 | 13.1 | 9.4 | 17.6 | 10.9 | 35.6 | 19.0 | 23.1 | 18.5 | 9.3 |
| % P | 0.9 | 1.3 | 0.4 | 1.4 | 1.2 | 2.5 | 2.1 | 1.4 | 1.1 | 1.7 | 1.5 | 1.8 | 1.1 |
| % MP | 2.5 | 4.2 | 6.0 | 5.8 | 4.8 | 5.8 | 9.4 | 8.0 | 2.7 | 6.3 | 6.4 | 5.2 | 6.8 |
| % DMP | 3.3 | 6.6 | 9.5 | 9.6 | 8.0 | 8.8 | 10.3 | 9.0 | 0.1 | 3.9 | 5.1 | 3.2 | 12.4 |
| % D | 1.7 | 1.8 | 0.6 | 1.5 | 1.4 | 2.2 | 2.6 | 1.8 | 2.8 | 4.0 | 3.0 | 5.6 | 1.4 |
| % MD | 4.7 | 8.9 | 5.3 | 11.0 | 14.9 | 13.5 | 8.5 | 10.3 | 6.0 | 7.3 | 11.7 | 10.4 | 13.1 |
| % DMD | 9.4 | 15.4 | 25.3 | 21.9 | 29.9 | 27.7 | 22.6 | 25.2 | 8.5 | 11.8 | 20.5 | 12.6 | 32.7 |
| % TMD | 6.1 | 9.3 | 33.1 | 17.5 | 14.0 | 11.4 | 9.8 | 10.3 | 4.3 | 5.8 | 10.8 | 4.4 | 18.4 |
| % N | 71.2 | 52.8 | 20.2 | 31.4 | 25.9 | 28.3 | 34.8 | 34.0 | 74.7 | 59.5 | 41.0 | 57.0 | 13.9 |
| % P | 6.6 | 12.1 | 15.9 | 16.8 | 14.0 | 17.1 | 21.8 | 18.4 | 13.9 | 11.9 | 13.0 | 10.2 | 20.3 |
| % D | 21.8 | 35.4 | 64.3 | 51.9 | 60.2 | 54.7 | 43.4 | 47.6 | 21.6 | 28.6 | 46.0 | 33.0 | 65.9 |

higher, reaching up towards 1000 μg/L in March. The three dominating peaks in the beginning of gas chromatogram (Fig. 3) originate from the dispersant (Finasol OSR 2) and were identified by gas chromatography–mass spectrometry to be the straight-chain alkanes $C_{10}$, $C_{11}$, and $C_{12}$. The main treatment with the dispersant was undertaken March 10 and was repeated with smaller amounts on March 19 and April 24. The three dominating alkanes from the dispersant could be traced until June together with small but significant amounts of oil.

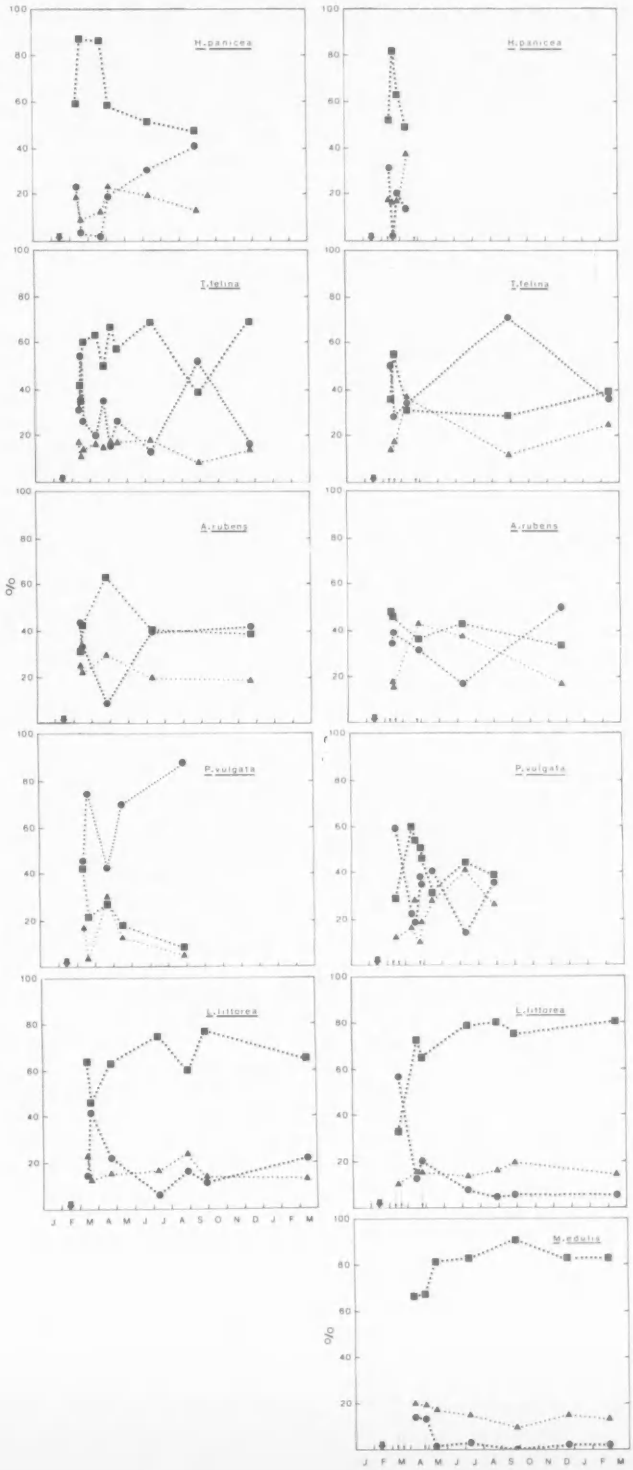
The contents of the aromatic hydrocarbons in the water (Table 1) show a very irregular picture, most likely due to variables such as temperature, wind, waves, tide, and current. The only clear tendency was that during the first months after the spill the water from the location where chemical dispersant was used (B) contained the most aromatics. The relative amounts of naphthalenes, phenanthrenes, and dibenzothiophenes also showed considerable variability, although the trend during the first half year was that the naphthalenes decreased from relative amounts of ~60% to ~20%, while the dibenzothiophenes increased from ~20–30% to ~60%, and the phenanthrenes stayed constant at ~10–20%. This reflected roughly the pattern found in the oil left on the shore. A closer examination of the different compounds with various alkyl substitutions revealed that the dimethyl derivatives were the dominant ones for all three classes of compounds.

Blank tests, carried through the complete procedure for analysis of the aromatics in the animals, showed that traces of the aromatics were introduced to the sample during the workup in amounts equivalent to ~0.03–0.05 μg/g of biological sample. Analysis of the organisms from the reference location showed minimal amounts of the aromatics, ranging from the blank values to ~0.1 μg/g. The occasional samples with levels slightly above the blank value may have arisen from traces of dissolved aromatics that could have reached the reference site, although no oil was observed on the surface. On this basis, together with experience from analyses of organisms from unpolluted areas elsewhere on the Norwegian coast, the conclusion is that the presence of naphthalene, phenanthrene, and dibenzothiophene and their alkyl derivatives in animal tissue indicates that the animals have been polluted by mineral oil.

Figure 4 shows the total amounts of the aromatics in the animals as a function of time. At the start of the investigation, 1 mo after the spill occurred, all the species contained significant quantities of the aromatics with a maximum of 28 μg/g wet tissue in the mussels from location B. Relative to the concentrations found in water (Table 1), the accumulation factors ranged up to an excess of 1000. Similar factors have been demonstrated earlier, under field conditions (Ehrhardt and Heinemann 1975) and in the laboratory (Fossato and Canzonier 1976; Stegeman and Teal 1973).

Due to the uncontrolled parameters in the present study (e.g. temperature, wind, waves, tide, current) the results were very variable. However, a few trends were apparent. The high initial amounts of aromatics persisted in the animals for the first 2 wk, but from April on the amounts decreased rapidly. During the next 2–3 mo the concentrations reached low but significant levels that were retained until the end of the year. Of the species analyzed 1 yr after the spill, small amounts of aromatics were detected in the mussel (February 77: 1.4

FIG. 5. Relative amounts of naphthalenes (circles), phenanthrenes (triangles), and dibenzothiophenes (squares) in the tissue samples. The left-hand graphs represent data from location A; the right, location B. Arrows on the abscissa as in Fig. 4. Note: no samples of *M. edulis* from location A.


H.panicea
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P.vulgata
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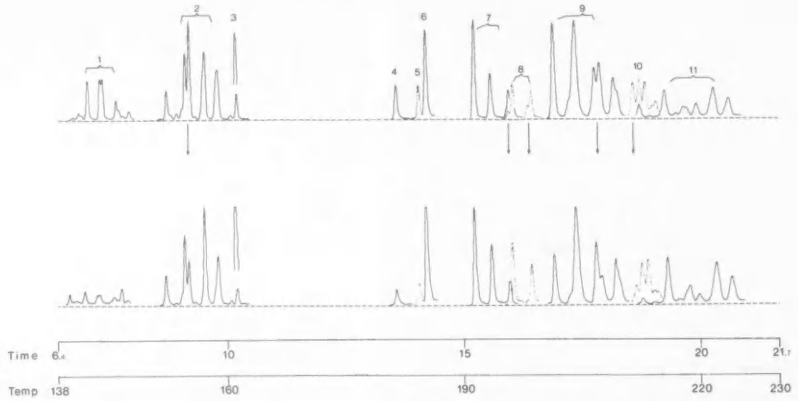


FIG. 6. Mass fragmentograms of extracts from tissue of anemones sampled on location A on April 22 (*top*) and July 9 (*bottom*). Arrows indicate differences in the patterns of the isomers. Peaks are as follows: 1 — dimethylnaphthalenes, 2 — trimethylnaphthalene, 3 — fluorene (internal standard), 4 — dibenzothiophene, 5 — phenanthrene (dotted line), 6 — anthracene (internal standard), 7 — methyldibenzothiophene, 8 — methylphenanthrene (dotted line), 9 — dimethyldibenzothiophene, 10 — dimethylphenanthrene (dotted line), 11 — trimethyldibenzothiophene.

$\mu$g/g at location B) and in the periwinkle (March 77: 0.6 $\mu$g/g at location A and 0.8 $\mu$g/g at location B), while amounts above background were not apparent in the limpet, anemone, and sponge. Starfish and crabs were not collected in 1977.

In planning this investigation two aspects were of special interest: a comparison of the location where chemical dispersant was used in the cleanup with the location where the oil along the shore was removed by physical methods only, and a comparison of the different species with respect to uptake and discharge of aromatics.

The obvious differences between the two locations in amounts of hydrocarbons in the water during the first months after the spill (Table 1) were not reflected in the animals. This does not necessarily imply that the chemically dispersed oil was not as easily taken up by the animals as naturally dissoluted hydrocarbons. The present findings may well have been results of one or more of the uncontrolled factors together with the fact that water and animals were not sampled at exactly the same spots. However, this aspect should be given closer attention under controlled laboratory conditions.

The data in Fig. 4 might suggest differences between the species with respect to uptake and discharge of the aromatics, but when all the variables in the field are taken into consideration, the evidence is not conclusive. A few facts were obvious. The crabs contained less aromatics than the other species. This most probably resulted from the higher mobility of the crabs rather than lower uptake. In each crab the hepatopancreas contained higher amounts than muscle tissue.

The limpets had taken up smaller amounts of aromatics than the periwinkles, although both live in the intertidal area and have similar feeding habits. For both species the third treatment with dispersant in location B resulted in an increase in the content of aromatics. There also appeared to be an increase during the summer explainable by a higher activity of the animals.

The relative amounts of the various naphthalenes, phenanthrenes, and dibenzothiophenes in oil and animals sampled in the beginning of the investigation are shown in Table 2. The 1st mo of weathering of the oil had resulted in a relatively larger disappearance of the naphthalenes than of the other aromatics, reducing their share of the total from 71 to 53%, with a corresponding increase in the phenanthrenes and dibenzothiophenes. Naphthalene itself had vanished completely, and the monomethyl derivative had also decreased drastically. Among the phenanthrenes and dibenzothiophenes there was also a shift towards higher alkyl substitution.

Totally, however, the aromatics had suffered a greater loss than the oil as a whole. As mentioned above, the weight reduction of the oil was 50%, and the share of the aromatics had been reduced from 1.22% in the original oil to 0.76%, meaning that close to 70% of the aromatics had disappeared.

In the animals there was a distinct difference between the periwinkles and limpets on one hand and the animals living below the tidemark on the other. The composition of the aromatics found in the tissue of the former two resembled that of the oil on the shore more closely than did that of the other animals. This was probably due to the difference in exposure and availability of the oil.

The relative composition of naphthalenes, phenanthrenes, and dibenzothiophenes in the tissues of the

various animals is given in Fig. 5. Although the picture seems somewhat confusing it can be seen that the dibenzothiophenes were dominant in the majority of the tissue samples. The limpets from location A were the only significant exceptions. The mussels and the periwinkles especially, and to some extent the sponges, demonstrated a preferential retention of the dibenzothiophenes.

The sulfur-containing aromatics have only been given marginal attention to date (Lake and Hershner 1977; Warner 1975). The present findings suggest that these compounds may play a more important role in the pollution of marine animals than their amount in the polluting oil implies.

The mass fragmentograms of the extract of the tissue of anemones sampled at location A on April 22 and on July 9 are shown in Fig. 6. The total amounts of aromatics were 7.5 and 3.6 $\mu$g/g wet weight, respectively. Differences in the patterns in the two fragmentograms are obvious. In the sample from July the dimethylnaphthalenes had decreased relative to the trimethyl derivatives. Naphthalene and methylnaphthalene were not present in significant amounts in any of the samples. The relative sizes of several other peaks representing different isomers had changed from April till July. These changes might be difficult to explain by chemical–physical factors alone, suggesting that physiological mechanisms are active in the release of the aromatic hydrocarbons from the tissue. This is a topic for further investigation.

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# Preliminary Ecological–Genetic Comparison Between Unperturbed and Oil-Impacted *Urosalpinx cinerea* (Prosobranchia: Gastropoda) Populations: Nobska Point (Woods Hole) and Wild Harbor (West Falmouth), Massachusetts[1]

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COLE, T. J. 1978. Preliminary ecological–genetic comparison between unperturbed and oil-impacted *Urosalpinx cinerea* (Prosobranchia: Gastropoda) populations: Nobska Point (Woods Hole) and Wild Harbor (West Falmouth), Massachusetts. J. Fish. Res. Board Can. 35: 624–629.

Catastrophes, whether natural or man-made, impinge on populations of organisms by increasing mortality, thus reducing population numbers. Recent theoretical studies indicate that demographic and genetic changes may result from such events. The 1969 oil spill at Wild Harbor offered an opportunity to obtain empirical data on this relationship between ecology and genetics. The organism studied was the oyster drill *Urosalpinx cinerea* (Say, 1822). Monthly samples (April to September) were collected for each of 3 yr (1973–75) from the recolonizing population at Wild Harbor and from a long-standing population at Nobska Point. Genotypes at two allozymic loci (ODH and LAP) were determined. Demographic information was obtained by mark–recapture experiments during 1973–75. Analyses of population dynamics indicated that a reasonably functioning population has been reestablished at Wild Harbor. However, genetic structure (as revealed by genotypic distributions) of Wild Harbor drills exhibited generally greater year-to-year variation than did Nobska organisms. This may have resulted from genetic change caused by gene migration from different coadapted gene complexes and from stochastic "founder" effects. More information on the interdigitation of ecology and genetics is needed, however, before implications of these findings are fully realized.

*Key words:* electrophoresis, genetics, oiled population, oyster drill, petroleum, *Urosalpinx cinerea*

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Qu'elles soient naturelles ou causées par l'homme, les catastrophes réduisent les populations d'organismes en augmentant la mortalité, causant ainsi une diminution des nombres que renferme la population. Des études théoriques récentes indiquent que de tels événements peuvent entraîner des changements démographiques et génétiques. Le déversement de pétrole de 1969 à Wild Harbor fournit l'occasion de recueillir des données empiriques sur cette relation entre l'écologie et la génétique. L'organisme étudié fut le perceur d'huître, *Urosalpinx cinerea* (Say, 1822). Nous avons prélevé des échantillons mensuellement (avril à septembre) durant chacune de 3 a (1973–75) à même la population en voie de recolonisation à Wild Harbor et à même une population établie depuis longtemps à Nobska Point. Nous avons déterminé les génotypes à deux loci allozymiques (ODH et LAP). Des marquages effectués de 1973 à 1975 fournirent les renseignements démographiques. Les analyses de dynamique de population indiquent qu'une population fonctionnant raisonnablement bien s'est réétablie à Wild Harbor. Cependant, la constitution génétique (telle qu'indiquée par les distributions génotypiques) des perceurs d'huître de Wild Harbor accusent en général une plus grande variation, d'une année à l'autre, que les organismes de Nobska. Cela peut être le résultat d'un changement génétique causé par la migration de gènes depuis des complexes différents de gènes coadaptés et par des effets stochastiques de « fondateur ». On a besoin d'une information plus complète sur l'interaction entre l'écologie et la génétique avant de pleinement réaliser les implications de ces découvertes.

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ACCIDENTAL spills of petroleum hydrocarbons into the marine environment usually result in the death of oil-fouled organisms (Sanders et al. 1972). Although some individuals may survive to reinitiate population growth, most species depend on recruitment of larval or juvenile individuals dispersing from other populations. A few species, i.e. the oyster drill *Urosalpinx cinerea*, lack an effective dispersive stage, so the potential for migration of individuals between disjunct populations on physically separated substrates is greatly reduced.

In 1969 a spill of No. 2 fuel oil occurred near the shores of West Falmouth, Mass. The aftermath of the spill has been well documented and investigated (Sanders et al. 1972; Michael et al. 1975). Particularly affected were the molluscs of this region (Blumer et al. 1970), especially those of the rocky intertidal community (H. Sanders, F. Grassle, G. Hampson, and G. Souza, personal communication). This offered an opportunity to study the genetic structure of a population recovering from a catastrophic decline in numbers, compared with a reference population in an area unaffected by the oil spill. Recent theoretical studies have indicated extensive genetic change may be associated with demographic catastrophes (Charlesworth 1973, 1974; Charlesworth and Giesel 1972).

The organism chosen (*U. cinerea*) is the major barnacle-bivalve predator of the temperate western Atlantic rocky intertidal of the Virginian and Carolinean provinces (Wood 1968). Development is entirely within an attached egg-capsule and individuals hatch as juveniles. There is no pelagic stage and little potential for gene flow between disjunct populations. Adults exhibit little horizontal migration, and locomotion is poor on other than hard surfaces (Federighi 1931). Dispersal occurs by rafting or through the agency of man (Carriker 1955). There is some evidence that disjunct populations of *U. cinerea* constitute a series of distinct physiological races (Stauber 1950; Shick 1972). *Urosalpinx* does exhibit a pronounced seasonal vertical migration (Carriker 1954). During summer it is intertidal, but as water temperatures decline in the fall, *U. cinerea* migrates downwards to subtidal hibernation sites at rock–sediment interfaces. Here the animals bury themselves in the substrate, with only the siphon above the surface, and overwinter in this position (Carriker 1954; Carriker and Van Zandt 1973). As water temperatures rise to 10°C in spring, the animals begin to move slowly about (Carriker and Van Zandt 1973). Copulation takes place as the animals leave their overwintering sites and ascend into the intertidal in early summer. Females spawn gregariously at subtidal sites during their upward migration; on Cape Cod this typically occurs in early May.

## Methods and Materials

The reference drill population was at Nobska Point (Vineyard Sound, Woods Hole, Mass.). This population inhabits a stonework jetty and is surrounded by sand. Barnacles are the principal prey. The Wild Harbor population also lives on a stonework jetty surrounded by sand. This site, part of the Buzzards Bay hydrographic system, experiences less wave action than does Nobska. Salinity and thermal regimes are comparable (T. Cole unpublished data). Food for Wild Harbor *U. cinerea* is mostly barnacles, but some bivalve species are available.

Samples of 100 snails were collected monthly from each of these sites for 3 yr (1973–75) during the active period for Cape Cod *U. cinerea* (April–September). These animals were frozen at −20°C until used for the electrophoretic analysis described below. Upon thawing, animals were measured [shell length, shell width (maximum diameter), aperture length, and spire height] with vernier calipers (0.1 mm precision). Gonadal texture and coloration plus the presence or absence of ancillary reproductive structures were used to determine sexes (Fretter and Graham 1962).

No sampling was attempted during the hibernation period (October–March) to avoid introducing additional and artificial mortality. Hibernating snails, if dislodged at winter temperatures, have difficulty reattaching and reorienting themselves (Carriker and Van Zandt 1973).

Concurrently a mark–recapture program was conducted at these locations to obtain demographic information. Tagging procedures were adapted from Frank (1965; personal communication). Recaptures were monthly during spring low tides from April to September (1973–74). At least one low tide period per recapture episode was spent searching adjacent rock structures and beaches for marked snails. Beginning in April 1975, all marked snails collected were returned to the laboratory for electrophoretic genotyping.

Snails were genotyped by vertical starch-gel electrophoresis in the discontinuous histidine–sodium citrate (pH 7.0) buffer system of Brewer (1970). Runs were for 4 h at 3–4°C, after which the gels were removed, sliced, and stained. Breeding studies have shown that the octanol dehydrogenase (ODH) and leucine aminopeptidase (LAP) loci of *U. cinerea* foot muscle exhibit simple Mendelian inheritance (T. Cole unpublished data). Both of these enzymes were visualized after electrophoresis by the methods of Ayala et al. (1972).

## Results

No significant differences in shell-length frequencies were found among the samples for any month or for either population ($G_{II}$ test; $P < 0.9$). Modes for the two populations were nearly the same. Recruitment of juveniles began in late May and peaked in June.

Data from recapture studies at both Wild Harbor and Nobska were analyzed by Jolly's (1965) stochastic method for estimation of population dynamics. A more detailed presentation of these results is in preparation. Jolly estimates of population numbers at Wild Harbor were similar to those from Nobska drills in their monthly pattern of fluctuation and in absolute value. Population numbers were highest in May–June (reflecting recruitment), then declined steadily to September. April numbers were not significantly different from those of September (*t*-test; $P < 0.9$). Population growth during the 2 yr of study (1973–74) was negligible for the two populations. However, numbers of Wild Harbor males

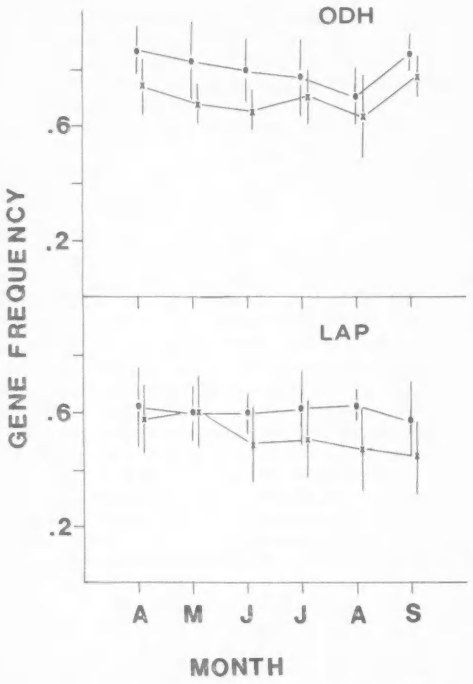


FIG. 1. Slow-allele frequencies at the ODH and LAP loci for Nobska and Wild Harbor populations. Data from the sexes and from the 3 yr of sampling were pooled and plotted as means (±1 SD). Months where significant heterogeneity ($G_H$ test; $P < 0.05$) among the estimates was detected are indicated in the text. O, Nobska; X, Wild Harbor.

and females were similar, while there were more females than males at Nobska.

The ODH and LAP loci each have two common alleles segregating (T. Cole unpublished data). A third allele for LAP was found in heterozygous combination in two Wild Harbor individuals. In both systems the term "fast" was applied to the protein band that migrated the farthest from the origin under standardized electrophoretic conditions; the other, slower migrating band, "slow". The frequency of the slow allele was selected as the standard for each locus.

Significant heterogeneity ($G_H$ test; $P < 0.05$) among gene frequency estimates from the different years was found for the ODH locus for May and July females and for July males at Nobska, and for April females and for May males at Wild Harbor. At the LAP locus, July and September samples of Wild Harbor females were also heterogeneous. No significant variability was found for LAP in Nobska samples. Monthly samples with significant heterogeneity in year-to-year gene frequencies were pooled by excluding the most outlying estimate. Male and female values were also pooled where possible. A more complete description will be presented elsewhere (T. Cole unpublished data).

Mean frequencies of the slow ODH allele among Nobska drills fluctuated between 0.897 (May) and 0.700 (August). At Wild Harbor, high and low points were 0.771 (September) and 0.620 (August). Generally the pattern was the same for both populations with high values in April and September and low values in midsummer (Fig. 1). Mean frequencies of the LAP slow allele at Nobska fluctuated between 0.610 (April) and 0.643 (August). At Wild Harbor, LAP slow allele frequencies trended downward from a high of 0.597 (May) to a low of 0.481 (September). Wild Harbor drills did not parallel Nobska drills closely at this locus (Fig. 1).

Deviations from expectations based on the Hardy–Weinberg distribution were calculated from the genetic data. This statistic reveals microevolutionary changes within populations by comparing observed zygotic proportions with those expected from calculated gametic proportions (Wallace 1968). The calculations used pooled data, except where significant heterogeneity was noted.

The comparison of this statistic from Nobska and Wild Harbor data indicated that among Nobska drills a temporal pattern was discernible for each locus and was similar for both sexes (Fig. 2). This was not observable for Wild Harbor snails. Numbers of ODH heterozygotes at Nobska dropped markedly in May, then steadily increased through June and July to slight surpluses in August. Another drop in heterozygotes occurred in September. LAP heterozygotes at Nobska increased from a marked depression in April to lesser deficiencies in May and June. A slight drop in heterozygote numbers occurred in July, followed by an increase in August with a similar drop in September (Fig. 2).

Wild Harbor ODH heterozygotes were as deficient in number as Nobska snails but differed in monthly pattern. Wild Harbor males and females at the ODH locus exhibited dissimilar patterns. At the LAP locus, little concordance between Wild Harbor sexes or with Nobska drills was discernible. These results are being further analyzed with data from the recapture experiments.

To dissect further the differences in distribution of heterozygous individuals between the two populations, the samples were subdivided into five, numerically comparable, size-classes: juveniles ($\sim \leq 14$ mm shell length; not abundant in April and September samples), 15–17 mm (excluding nonsexable animals), 18–19 mm, 20–21 mm, and $\geq 22$ mm snails. Percentages of heterozygous individuals within each of these shell-length classes were then calculated for each year, and means (±1 SD) plotted.

At the ODH locus among Nobska drills, little variation in heterozygote distribution among size-classes was observed among the 3 yr (Fig. 3). July and September samples were the most variable. For Wild Harbor, only May and August samples exhibited as little variation as Nobska. At the LAP locus, heterogeneity inherent

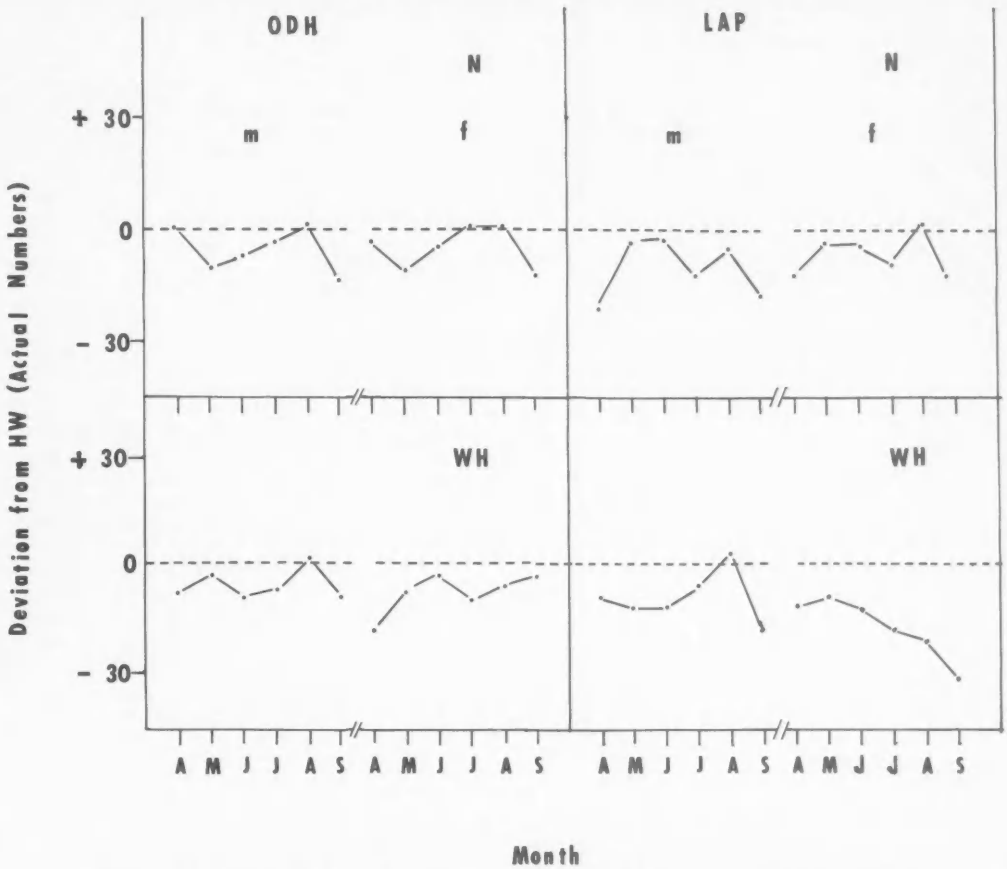


FIG. 2. Deviations in numbers of observed ODH and LAP heterozygotes from those expected based on Hardy–Weinberg (HW) distribution. Expected values calculated from pooled data. Instances where significant heterogeneity in gene frequency existed were pooled by excluding the most outlying point. N, Nobska; WH, Wild Harbor; m = male; f = female.

in the monthly samples was comparable for the two populations (Fig. 4). The greatest degree of variation was observed among July and September samples for Nobska and Wild Harbor.

## Discussion

Comparisons of shell-length frequencies among years at Wild Harbor indicate that by 1973 this population had achieved a size structure not significantly different from an unperturbed Cape Cod *Urosalpinx* population. Franz (1971) found that an individual *U. cinerea* achieves 70–80% of its ultimate shell size within its second summer. Since the September 1969 oil spill at Wild Harbor three growing seasons would have been available to the recolonizing population before experimentation began. Michael et al. (1975) noted that snails of the Wild Harbor rocky intertidal bordering the boat basin (site of the greatest concentration of petroleum hydrocarbons in sediments) seemed unaffected by close proximity to this pollution, while species of the soft-bottom community were severely affected. Thus, three growing seasons would be sufficient for a *U. cinerea* population to assume its typical size spectrum. Age structure perhaps would not be at equilibrium, but at present, age analysis by shell growth lines is not reliable with marine gastropods.

Comparisons of genetic structure of these two populations reveal that both exhibit the same temporal pattern of ODH slow allele frequencies and generally equal deficiencies of heterozygotes in the monthly samples. The monthly pattern of heterozygote deficiency among Nobska drills is similar for both sexes, while Wild Harbor males and females differ from each other and from Nobska. Wild Harbor drills show greater overall variability than Nobska drills in the percentage of

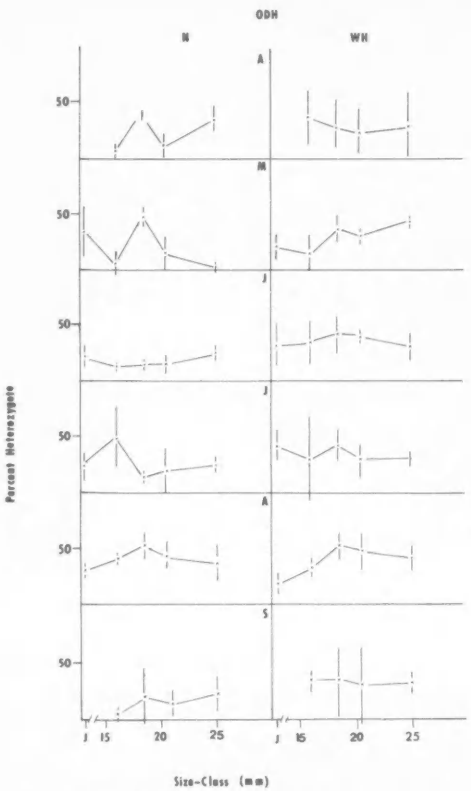


FIG. 3. Percent ODH heterozygotes per size-class present in monthly samples of Nobska and Wild Harbor *Urosalpinx cinerea*. Size-class division is given in text. Plotted are means (±1 SD) for 3 yr (1973–75) of monthly samples. Male and female data were pooled.

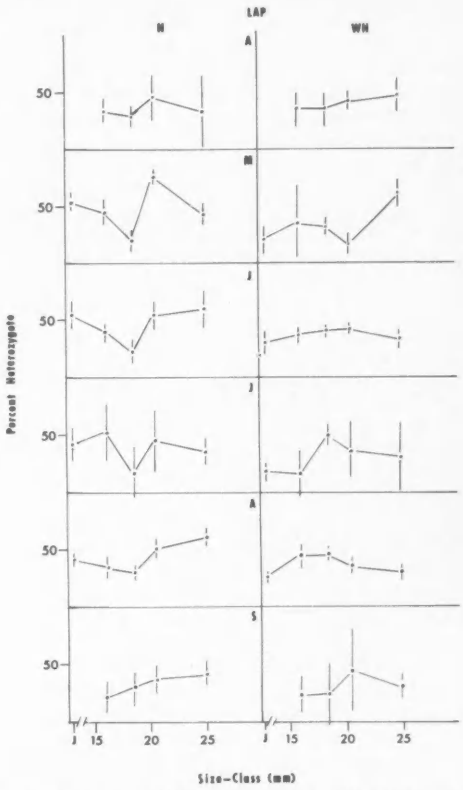


FIG. 4. Percent LAP heterozygotes per size-class present in monthly samples of Nobska and Wild Harbor *Urosalpinx cinerea*. Details as in Fig. 3.

heterozygotes per size-class observed among the monthly samples, especially for the ODH locus.

Factors that could produce changes in genetic structure are incorporation of migrant genes, stochastic changes in frequencies of existing genes inherent in severe reductions of population size (founder effect), and a changed ecological environment. The relative contributions from these components are difficult to determine. Additionally, analyses of genotypic fitnesses as suggested by Charlesworth (1973, 1974; Charlesworth and Giesel 1972) now in progress indicate that the Wild Harbor population had not reestablished a stable age distribution during the tenure of study. This was affecting genetic structure through changed ($1_x$) ($m_x$) functions (T. Cole unpublished data).

*Urosalpinx cinerea* individuals have a maximal life span estimated at 7–9 yr after initial reproduction (Comfort 1957; T. Cole unpublished data). Older drills tend to be larger, and size and fecundity are positively correlated in marine gastropods (Spight et al. 1974). This suggests that the genetic structure of the Wild Harbor population will only slowly approach equilibrium. However, since at present the interrelationship between ecology and genetics is so imperfectly understood, the implications of these findings for the future of this population are unknown.

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# Alterations in Growth and Physiology of Soft-Shell Clams, *Mya arenaria*, Chronically Oiled with Bunker C from Chedabucto Bay, Nova Scotia, 1970–76[1,2]

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GILFILLAN, E. S., AND J. H. VANDERMEULEN. 1978. Alterations in growth and physiology of soft-shell clams, *Mya arenaria*, chronically oiled with Bunker C from Chedabucto Bay, Nova Scotia, 1970–76. J. Fish. Res. Board Can. 35: 630–636.

Two populations of soft-shell clam, *Mya arenaria*, one from a chronically oiled lagoon (since the *Arrow* oil spill in 1970) and the other from a nonoiled lagoon, were compared as to population structure, growth, and metabolism. The oiled lagoon sediments contained up to 3800 μg/g oil (UV determination), and clams up to 200 μg/g hydrocarbon in their tissues (fluorescence). The oiled population of clams differed from the nonoiled population in lower total numbers with fewer mature adults, a 1–2-yr lag in tissue growth, a lower shell growth rate, and a reduced carbon flux with a lower assimilation rate. Results are interpreted to indicate that the recovery potential of *M. arenaria* in these oiled sediments is low and that these oiled populations remain under continued stress 6 yr after the *Arrow* spill.

*Key words:* physiology, petroleum, growth, carbon flow, clams, recruitment

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Nous avons comparé la structure de population, la croissance et le métabolisme de deux populations de mye, *Mya arenaria*, l'une provenant d'une lagune chroniquement affectée par le pétrole (depuis le déversement de l'*Arrow* en 1970) et l'autre, d'une lagune non touchée. Les sédiments de la lagune polluée contiennent jusqu'à 3 800 μg/g de pétrole (déterminé par UV) et les myes, jusqu'à 200 μg/g d'hydrocarbures dans leur tissu (fluorescence). Les populations de myes affectées par le pétrole diffèrent des populations non affectées en ce celles-là comptent moins d'individus et un nombre moindre d'adultes mûrs, subissent un décalage d'un ou de deux ans dans la croissance du tissu, un rythme plus lent de croissance de la coquille et ont un flux de carbone réduit avec vitesse d'assimilation moindre. D'après ces résultats, nous croyons que le potentiel de rétablissement de *Mya arenaria* dans ces sédiments pollués par le pétrole est faible et que ces populations affectées sont toujours soumises à un stress, six ans après le déversement de l'*Arrow*.

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SOFT-SHELL clams, *Mya arenaria*, are particularly subject to deleterious effects from oil spills because of a number of ecological, morphological, and behavioral characteristics. They tend to live in fairly fine-grained sediments in low-energy environments and once these sediments are oiled the oil will persist for long periods of time (Blumer and Sass 1972; Vandermeulen and Gordon 1976). Because of their shell morphology the clams cannot completely close their shells; thus the mantle and gill surfaces are always exposed to sediments and interstitial water. Because these animals obtain their food from the boundary layer of water in intimate contact with the sediment surface, oil leaching out of these sediments will be readily available to the clams before it becomes dispersed.

The impact of oil on clam growth and physiology and its persistence over time has been well documented. A postspill study of *M. arenaria* oiled by No. 6 fuel oil in Casco Bay, Maine, in 1972 showed that metabolism and growth were reduced in oiled clams for as long as 3 yr after the spill (Gilfillan et al. 1976, 1977a). Further investigations of a spill of light refined oil at

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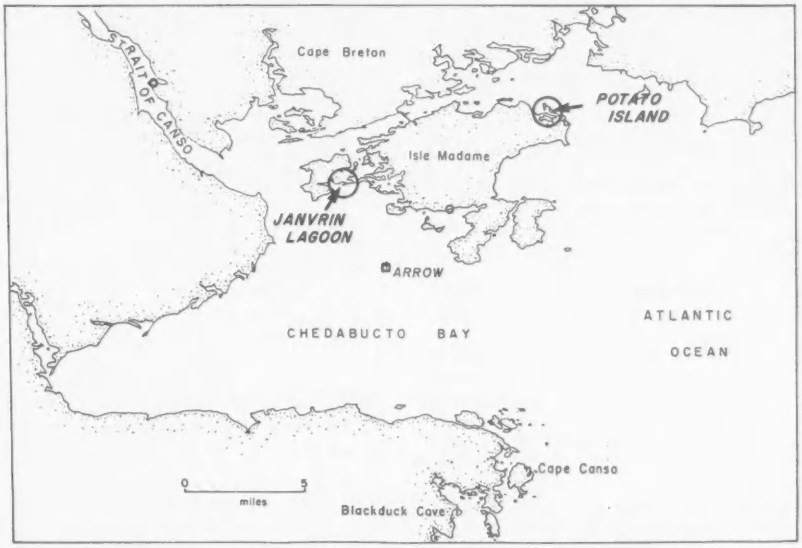


FIG. 1. Chedabucto Bay, N.S., area showing site of the *Arrow* grounding and location of the two lagoons sampled in this study. Janvrin Lagoon was heavily oiled after the tanker sinking, while the Potato Island lagoon remained clean.

Searsport, Maine, have shown that reduced growth, survival, and recruitment in *M. arenaria* were apparent up to 5 yr postspill (Gilfillan et al. 1977b; Mayo et al. 1978). Thomas (1973, 1978) found *M. arenaria* populations in oiled Chedabucto Bay lagoons to be in continuous decline after the sinking of the *Arrow* and its Bunker C spill in 1970, and indications were that this condition continued through 1975.

Little is known of the long-term recovery potential of oiled clams, and of the interrelationship of population recovery and long-term physiological stress. In the present study we provide data on carbon flux and shell and tissue growth, and on population structure and recruitment of *M. arenaria* from chronically oiled sediments 6 and 7 yr postspill. Results are discussed in terms of population recovery potential during chronic oil exposure.

## Materials and Methods

Janvrin Lagoon and Potato Island Lagoon were chosen as representative of an oiled and a nonoiled area, respectively. These lagoons are part of the Isle Madame, Chedabucto Bay, shoreline, and are approximately 11 km apart on opposite sides of the island. They are part of the same shoreline system and are fed by the same water system passing around the island through Lennox Passage (Fig. 1). Both lagoons are open at one end by a narrow passage to incoming and outgoing tide, and are of approximately the same area. In addition the gross morphology of clam-bearing sediments is similar in both lagoons. There has never been commercial clam harvesting at either location, although some digging for personal use occurs occasionally. Both are submerged to similar depth at high tide. Janvrin Lagoon still retains significant traces of stranded Bunker C oil along its shoreline and within the bottom sediments.

Clams were taken from three stations in Potato Island Lagoon and from six stations in Janvrin Lagoon.

### Sediment and Tissue Hydrocarbons

Two to twenty grams of sediment of known weight was slurried with pentane-extracted distilled water (5 mL) in a tared 50 mL glass centrifuge tube, and then extracted twice with 10 mL redistilled pentane on a Burrell wrist-action shaker (20 min, maximum setting). Phase separation was obtained by centrifugation (IC centrifuge, Model CL, 10 min). The pentane supernatants were pooled after each extraction, reduced to 5 mL by nitrogen evaporation, and brought back to 10.0 mL by adding pentane.

Hydrocarbon concentrations in the pentane extract were determined fluorometrically ($\lambda_{ex} = 310$ nm, $\lambda_{em} = 374$ nm, Perkin-Elmer MPF-2A fluorescence spectrophotometer). Those from oiled sediments were corrected by subtraction for intrinsic background fluorescence.

Clams were removed from their shells and cleaned thoroughly to remove any adhering sediment or detritus. The tissues were rinsed with redistilled acetone to remove any external contaminating hydrocarbons and were allowed to air-dry for 15 min. These were then minced into tared glass tissue-grinders (Potter-Elvejlhem). Wet weights were recorded.

Minced tissue was extracted in 5 mL redistilled hexane (10 strokes), and the supernatant drawn off by pipette. Minced tissue was extracted twice in this manner. The pooled hexane extract was subsequently dried for 10 min with anhydrous extracted $Na_2SO_4$, centrifuged to remove any suspended solids, and concentrated to 10 mL by nitrogen evaporation (N-Evap, Organomation).

Hydrocarbon concentrations were determined by UV absorbance ($\lambda = 230$ nm, Pye-Unicam SP500 UV spectrophotometer). Synchronous fluorescence scans of sediment and tissue extracts were done by the method of Hargrave and Phillips (1975).

### Population Structure/Tissue and Shell Growth

To determine estimates of population size and composition in the two lagoons, all clams were taken from $\frac{1}{4}$ m$^2$ plots at each of the sampling stations. Plots were excavated to a depth of either 20 cm or to impervious clay, and sediments were sieved through a 2-mm mesh.

Specimens collected were freeze-dried, and shell length, tissue dry weight, and age were recorded. Age was determined by counting shell annuli.

Two independent determinations of age were made on each shell. The same two persons aged all shells; disagreements were rare. This procedure resulted in age-groupings that were remarkably homogeneous with respect to length and dry weight. Shell growth (width of annuli, millimetres) was determined for 1966–69 and 1972–75 by measuring the amount of shell growth in 11 and 12 year-class clams (1976) between these years. In this case age was determined by counting back from the 1976 partial growth ring.

### Physiological Measurements

Carbon flux was determined by measuring the amount of carbon respired versus the amount assimilated after ingestion of $^{14}$C-labeled algae (viz Gilfillan et al. 1976). All measurements were carried out at the ambient seawater temperature (ca. 13°C), in water taken from the appropriate lagoon.

Respiration rates were determined polarographically in a water-jacketed Plexiglas cell. Respiration rates were calculated as microlitres $O_2$ per milligram dry weight per hour on the linear portion of the respiration curves. Carbon respired was calculated as respiration times 0.43 (assuming an RQ of 0.8).

Filtration rates were determined by measuring the amount of $^{14}$C-labeled algae (*Dunaliella tertiolecta*) cleared from an algal suspension by feeding *M. arenaria*. Clams were placed in 4-L plastic beakers filled with glass-fiber-filtered seawater to which sufficient $^{14}$C-labeled algae had been added to yield a final chlorophyll $\alpha$ concentration of 2.5 $\mu$g chlorophyll $\alpha$/L.

The volume swept clear of particles ($V$) was calculated from the following relationship:

$$V = v(\ln C_0 - \ln C_t)t,$$

where $v$ is the beaker volume, $C_0$ and $C_t$ the initial and final radioactivity (disintegrations per minute per millilitre, dpm/mL), and $t$ is the duration of the measurement (hours). Filtration measurements were made over 3-h periods at 13°C.

Assimilation rates were calculated from algal and clam tissue radioactivity after feeding and a 24-h dose of unlabeled algae. After the 3-h filtration run the clams were transferred to seawater containing unlabeled algae for 24–30 h. Subsequently the clams were freeze-dried, weighed, and tissue radioactivity determined by liquid scintillation counting.

Assimilation rates were obtained from the ratio of dpm in clam tissues to dpm in algae volume consumed.

Table 1. Petroleum-hydrocarbon concentrations in sediments and clam tissues from Janvrin Lagoon (chronically oiled) and Potato Island Lagoon (nonoiled), Chedabucto Bay, N.S. Values shown are expressed as Bunker C equivalents, using *Arrow* Bunker C standard. Sediment-hydrocarbon concentrations were determined spectrofluorometrically, tissue hydrocarbons by UV absorption. Number of clams in each sample ($N$) = 5.

| | ($\mu$g/g dry weight sediment) | |
|---|---|---|
| Stn. | Janvrin Lagoon | Potato Island |
| | *Sediments* | |
| 1 | 714.24 | 0.838 |
| 2 | 644.73 | 0.98 |
| 3 | 3805.43 | 0.71 |
| 4 | 89.12 | |
| 5 | 1502.65 | |
| 6 | 87.15 | |
| $\bar{x}$ | | 0.84 |
| | *Clams* | |
| 1 | 305.46 ± 55.26 | 157.71 ± 50.48 |
| 2 | 349.53 ± 52.64 | 140.68 ± 47.27 |
| 3 | 231.14 ± 84.54 | |
| 4 | 190.99 ± 14.82 | |
| 5 | 252.16 ± 32.75 | |
| 6 | 272.89 ± 28.54 | |
| $\bar{x}$ | 267.03 ± 55.87 | 149.20 ± 46.18 |

Carbon budgets were calculated assuming the carbon concentration available in the algal suspension was 1000 $\mu$g C/L.

Respiration rates and filtration rates were expressed in terms of a standard specimen of 100 mg dry weight, using the linear relation between these two rate functions and the common logarithm of clam dry weight.

The following relationships were used to calculate carbon budgets for the nonoiled and oiled clams:

gross C intake = environmental C ($\mu$g/L) × volume filtered (L/hr);
assimilated C = gross C intake × assimilation ratio;
respired C = $\mu$L $O_2$ respired × 0.43;
C flux = assimilated C − respired C.

## Results

### Sediment and Tissue Hydrocarbons

Levels of petroleum hydrocarbons in sediment and clam tissues are shown in Table 1. All tissue and sediment samples examined from Janvrin Lagoon contained traces of oil, although there exists some variation. The Potato Island values reflect native UV absorption and fluorescence due to biogenic dynamics in sediment and clam tissues, and serve as background control values. Some sediment samples were visibly contaminated. Clam tissues were found to contain up to 200 $\mu$g/g tissue weight. Sediment samples contained up to 3800 $\mu$g/g sediment.

Synchronous fluorescence scans of Janvrin Lagoon

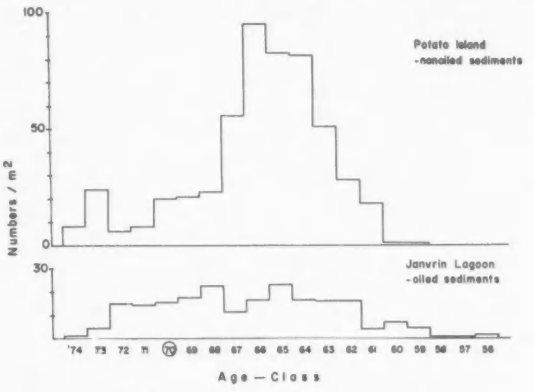


FIG. 2. Age-class distribution of *Mya arenaria* from Janvrin Lagoon and from Potato Island lagoon; 1970 (circled) was the year of *Arrow* grounding.

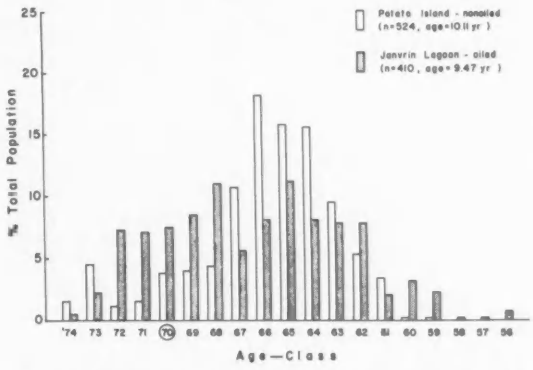


FIG. 3. Population composition of *M. arenaria* from the two lagoons. Circled age-class indicates year of *Arrow* sinking.

clam and sediment extracts were indistinguishable from those of weathered stranded Bunker C samples taken from the lagoon shoreline.

## POPULATION STRUCTURE

Results of the population analysis are shown in Fig. 2 and 3. Figure 2 shows the number of clams per square metre of sediment in each age-class, taken from three sites in Potato Island Lagoon and from six sites in Janvrin Lagoon. A total of 524 clams were collected from the Potato Island sites and 410 from the six Janvrin Lagoon sites.

Per square metre, the Potato Island population was much larger, especially in the 9-yr-old and over age-classes (Fig. 2). The median age for the Potato Island population, based on 524 individuals, was 10.11 yr. The Janvrin Lagoon population was much more sparsely distributed; no one group of age-classes predominates.

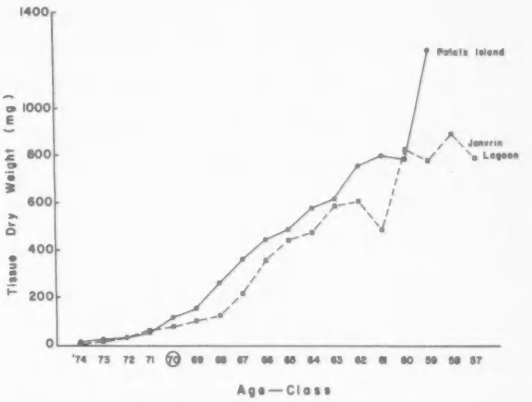


FIG. 4. Tissue growth of *M. arenaria* from a chronically oiled (Janvrin Lagoon) and a nonoiled reference site (Potato Island). Circled year-class indicates year of *Arrow* sinking.

Rather, the population distribution was generally flattened with approximately equal numbers of older and younger individuals. As a result, there was a proportionately larger component of younger, 4–8-yr-old age-classes, individuals in the Janvrin population (Fig. 3). This was also reflected in the slightly lower median age for the Janvrin population (9.47 yr).

## GROWTH

Tissue growth data from the two populations are summarized in Fig. 4. Generally, the two growth curves parallel one another. However, they diverge slightly in the 6-yr-old age-class and more widely in the older age-classes. As a result tissue growth in the Janvrin Lagoon population lags behind the Potato Island population by 1–2 yr.

Shell annular growth over the 1966–74 period is summarized in Table 2. Incremental shell growth in both populations was similar before 1970, averaging about 2 mm/yr. Around 1970 this growth rate declined in both populations. In the Potato Island population annuli growth decreased to 1.17 mm/yr, a reduction of 40.9%. However, in the Janvrin Lagoon population annular growth dropped markedly to an average of 0.825 mm/yr, a reduction in shell deposition of nearly 60%.

## CARBON FLUX

Results of carbon-flux determinations are shown in Table 3. Values of net carbon are based on an assumed carbon concentration during the feeding–assimilation experiment of 2000 μg/L. Although there is considerable variation clams from Potato Island Lagoon are clearly gaining carbon at a considerably greater rate than clams from Janvrin Lagoon. The C flux of the Potato Island population is maintained at a positive

TABLE 2. Shell growth, measured as increase in growth ring width, in 11- and 12-yr-old *Mya arenaria* in the 4-yr period before 1970 and after 1970.

| Stn. | Mean growth per year (mm/yr) Before 1970 | After 1970 | Percent 1970–74 growth of growth in 1966–70 |
|---|---|---|---|
| | *Potato Island* | | |
| 1 | 2.10 | 1.45 | |
| 2 | 1.74 | 0.90 | |
| 3 | 2.11 | 1.16 | |
| | 1.98 | 1.17 | 59.1% |
| | *Janvrin Lagoon* | | |
| 1 | 1.70 | 0.74 | |
| 2 | 2.10 | 0.88 | |
| 3 | 2.13 | 0.77 | |
| 4 | 1.94 | 1.14 | |
| 5 | 1.91 | 0.62 | |
| 6 | 2.33 | 0.80 | |
| | 2.02 | 0.825 | 40.1% |

TABLE 3. Carbon budgets for a nonoiled lagoon (Potato Island) and a heavily oiled lagoon (Janvrin Lagoon). Data expressed as g C/h per 100 mg 'standard' animal. Values shown are based on an assumed carbon concentration of 1000 $\mu$g C/L. $N = 5$.

| Stn. | Assimilated C | Respired C | C flux |
|---|---|---|---|
| | *Potato Island* | | |
| 1 | 67.73 | 15.53 | 52.20 |
| 2 | 12.60 | 42.66 | −30.06 |
| 3 | 22.80 | 23.46 | −0.66 |
| $\bar{x}$ | 34.38 | 27.18 | +7.16 |
| | *Janvrin Lagoon* | | |
| 1 | 23.10 | 30.43 | −7.13 |
| 2 | 14.33 | 26.10 | −11.81 |
| 3 | 6.37 | 30.95 | −25.60 |
| 4 | 7.66 | 24.80 | −17.15 |
| 5 | 2.27 | 23.03 | −20.76 |
| 6 | 2.74 | 44.60 | −41.87 |
| $\bar{x}$ | 9.41 | 29.98 | −20.72 |

level, whereas that of Janvrin Lagoon population is drastically reduced with a much lower C assimilation.

## Discussion

In discussing these results two fundamental problems must be taken into account: first, the validity of comparing two lagoon populations, however carefully selected, and second, the probability that these clam populations are at the limit of their geographic range and therefore not necessarily representative of the genus.

The first problem has really no solution. Two populations, however adjacent they be, will differ in some respects corresponding to the various physical and environmental factors of their respective areas. However, the lagoons selected do experience the same tidal and seasonal regimes and fluctuations and are of approximately the same size and depth profile. They are also part of the same water system and presumably receive the same plankton influx. Nonetheless, prior to 1970 clams grew at the same rate in both lagoons. As a result, one may assume that environmental conditions were similar in the two areas prior to 1970.

The second problem, that of these clam populations living at or near the limit of the species range, is real. Many organisms at the limit of their range do not necessarily show "normal" population characteristics and may well have particular physiological adaptations peculiar to their environment. However, the problem may be minimized by selecting as control a population as near as possible to the experimental population. Because of the closeness of the two populations and because of the fact that this species has a planktonic larval stage with wide dispersal, there is no reason to suspect that these two populations differ genetically.

### Growth and Population Structure

Growth, measured as shell growth ring incremental deposition, before 1970 appears to have been similar in Potato Island and Janvrin Lagoon. Presumably then the growth conditions in these two environments were also quite similar. However, shell annuli growth shows changes beginning in 1970, the year of the *Arrow* spill.

Both populations show some reduction in growth with increasing age. In the Potato Island population this reduction appears to be a characteristic associated with a population living near the limit of its range. Clams from Maine, 12 and 13 yr old, are growing at a constant rate per year (Dow and Wallace 1961). However, the slowest growing Maine clams are growing twice as fast as the clams from Potato Island. The reduction in growth at age 8 or 9 may therefore be normal in populations of *M. arenaria* from Chedabucto Bay. Even if it is not, the additional reduction in growth seen at Janvrin Lagoon is almost certainly a result of the oiling in 1970.

Reduction in growth due to environmental stresses has been well documented (Bohle 1972; Remane and Schlieper 1971; Thiesen 1968). Thus, if the population of *M. arenaria* in Janvrin Lagoon is being stressed by oil, other signs of stress, such as reduced survival, should also be evident. In fact, Thomas (1973) has presented convincing evidence for continuous decline of the clam population density in Janvrin Lagoon during the 3 yr after the *Arrow* spill. And on the basis of the samples collected in the present study, the population density in Janvrin Lagoon apparently remains well below the Potato Island clam density. Because no "before" data is available for Janvrin Lagoon, it is necessary to assume for comparison that the clam population there in 1970

was comparable with the present clam population at Potato Island. The physical similarities of the two areas combined with the fact that clam growth rates were very similar in both locations before 1970 strengthen this assumption. Comparison with Thomas' data (his table 1, 1973) suggests that reduction in the Janvrin Lagoon clam stocks continues to date. If our comparison of the Janvrin Lagoon and Potato Island clam populations is valid, then it appears that 6 yr after the spill the Janvrin stocks remain nearly 60% below normal population levels.

The age-class distribution at Potato Island suggests that in Chedabucto Bay successful, large-scale recruitment occurs infrequently, one such period being 1965–67. This is typical of bivalves, particularly when near the limit of their range (Coe 1956).

At present, both populations seem to be recruiting at roughly similar rates, and young clams at both locations are growing at similar rates. However, the older individuals are not. The reason for this difference is not known, but it may be related to the size or age of the clams. Clams will burrow 2–3 times their long diameter into the sediments (E. S. Gilfillan unpublished data), and young clams ~15 mm in length will burrow about 50 mm into the sediments. At Searsport, Maine, it was found that the uppermost layer of sediments (50–100 mm) was relatively clean of oil, presumably due to regular turnover by wave action, while large amounts of oil were present immediately below this clean surface layer (Gilfillan et al. 1977b). A similar observation has been made by Baker et al. (1978) in artificially oiled sediments at Milford Haven. Thus younger clams inhabit a cleaner surface layer, while the older, larger clams burrow down into the still oiled subsurface layer.

The data on hydrocarbon content of sediments in Janvrin Lagoon are for a composite sample. No sampling was done by depth, and there is no direct evidence that a low-hydrocarbon surface layer exists overlying a more heavily oiled subsurface layer. There are, however, strong biological indications that one does exist here as well. Clam spat appear to settle and grow equally well in both lagoons, the oiled and the nonoiled. But once they reach an age of ~5–6 yr or a length of ~15–20 mm, growth in Janvrin Lagoon is reduced, this reduction in growth more marked with increasing age (size). This apparent correlation between depth of sediment oiling and clam growth reduction with age needs further study.

For an understanding of the observed reduction in growth we must turn to the C flux measurements, for presumably the reduced carbon flux in the Janvrin Lagoon clams is related to the observed differences in growth between the Janvrin and the Potato Island populations.

## Carbon Flux

The outstanding point of the carbon-flux study is the marked difference in carbon flow between the control Potato Island population and the oiled Janvrin Lagoon population. Carbon-flux estimates do have inherent inaccuracies, primarily in the determination of the assimilation rates. First, these were uncorrected for respiration; however, the assumption must be made that recently ingested carbon is no more likely to be respired, nor excreted, than preexisting carbon. Also, carbon-flux determinations over a yearlong period have been shown to account for the annual increase in dry tissue weight in a *M. arenaria* population (Gilfillan et al. 1976). A second source of error in these particular carbon-flux determinations is that only one value was obtained at each of the stations. Carbon flux has been shown to be subject to variation in any given population (e.g. Gilfillan et al. 1976, 1977a, b), and "once only" measurements are not as reliable as multiple measurements.

Clearly, since there are clams in Janvrin Lagoon, these animals must be in calorific balance on an annual basis even though they were not at the time of sampling. It is common for populations of *M. arenaria* to not be in calorific balance at times throughout the growing season even though they are in calorific balance on an overall basis (Gilfillan et al. 1976, 1977a, b). However, at any given time if one growing area is better than another, the clams from the better growing area will show a higher value of carbon flow (less negative) even if both are not in carbon balance at the time. This appears to have been the situation at Potato Island and Janvrin Lagoon at the time of sampling.

The above notwithstanding, when comparing these two populations of soft-shell clams within the Chedabucto Bay area, the clams from Janvrin Lagoon are clearly gaining carbon at a much slower rate than those from Potato Island. The observed reductions in carbon flux can be traced to reduced filtration rates and reduced assimilation rates, coupled with slightly increased respiration rates.

This reduction in assimilation ratio appears to be a general response to environmental stress in mussels and clams and can be induced by a variety of factors. Lowered filtration rates and assimilation ratios have been observed in blue mussel, *Mytilus edulis*, exposed to seawater extracts of crude oil (Gilfillan 1975). A similar reduction in filtration rate and assimilation was found in a population of *Mya arenaria* oiled by a spill of No. 6 fuel oil (Gilfillan et al. 1976, 1977a).

More generally, it has been shown that reduction in scope for growth in mussels, a measurement analogous to carbon flux, is an efficient means of demonstrating the degree of stress applied to a population (Widdows and Bayne 1971; Bayne et al. 1973; Thompson and Bayne 1974; Bayne 1975; Bayne et al. 1977). As a result the lowered carbon flow seen in clams from Janvrin Lagoon is likely a manifestation of stress applied to the clam population by the presence of the oil there.

In summary, independent measures of carbon flux, shell and tissue growth, and survival indicate the continuous stress on the *M. arenaria* population of Janvrin

Lagoon by the oil from the *Arrow* there. The very marked change in carbon flux in these oiled bivalves together with the continued depression of clam stocks clearly indicates that Janvrin Lagoon remains at best a marginal habitat for these organisms. Given the fineness of the sediments and their oil-holding capacity as natural sinks in this low-energy environment, it is likely to remain that way for some time to come.

## Acknowledgments

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# Effects of Uptake and Discharge of Petroleum Hydrocarbons on the Respiration of the Soft-Shell Clam, *Mya arenaria*[1]

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STAINKEN, D. M. 1978. Effects of uptake and discharge of petroleum hydrocarbons on the respiration of the soft-shell clam, *Mya arenaria*. J. Fish. Res. Board Can. 35: 637–642.

A winter (4°C) spill condition was simulated in which young soft-shell clams, *Mya arenaria*, were exposed to subacute concentrations of No. 2 fuel oil-in-water emulsions for 28 d. Clams were exposed at the beginning of the experiment to single dose concentrations of 10, 50, and 100 ppm. Hydrocarbons were rapidly accumulated by clams within 1 wk after exposure. The accumulated hydrocarbons then decreased each week as the hydrocarbon content of the water decreased. Methyl substituted naphthalene isomers were the principal compounds accumulated and retained by the clams after 3 wk of oil exposure. A dose–response relationship was observed in the respiratory rates as measured by oxygen consumption ($QO_2$). Significant differences ($P = .05$) in respiratory rates were found in clams exposed to low concentrations of oil. The lowest concentrations of oil caused a doubling of the respiratory rates and greater oil concentrations caused a depression in rate. The respiratory rates of the clams exposed to low oil concentrations decreased as the hydrocarbon content of the water and clam tissues decreased, but remained significantly altered from the controls. Clams were transferred to an uncontaminated system for 14 d subsequent to the 28-d oil exposure to determine effects of depuration on the respiratory rate. During the depuration period, many of the hydrocarbons present in clam tissue were again found to be methyl substituted naphthalene isomers. During this period, the respiratory rates of the clams initially exposed to 10 ppm fuel oil emulsion remained significantly altered above the controls. The respiratory rates of all groups of oil-exposed clams remained altered from the controls, but the magnitude of difference tended to decline toward the controls. A dose–response narcosis may have been evident during this period.

*Key words: Mya arenaria*, respiration, No. 2 fuel oil, petroleum emulsion, petroleum accumulation, depuration

STAINKEN, D. M. 1978. Effects of uptake and discharge of petroleum hydrocarbons on the respiration of the soft-shell clam, *Mya arenaria*. J. Fish. Res. Board Can. 35: 637–642.

Nous avons simulé une condition hivernale (4°C) de déversement dans laquelle de jeunes myes communes (*Mya arenaria*) furent exposées à des concentrations subaiguës d'émulsions de fuel-oil nº 2 dans de l'eau durant 28 jours. Au début de l'expérience, les myes ont été exposées à des concentrations, par doses uniques, de 10, 50 et 100 ppm. Les hydrocarbures s'accumulent rapidement dans les myes en dedans d'une sem après exposition. Les hydrocarbures accumulés diminuent ensuite à chaque sem à mesure que la teneur de l'eau en hydrocarbures diminue. Les isomères naphtalène à méthyl substitué sont les principaux composés qui s'accumulent dans les coques et qui sont retenus après 3 sem d'exposition au pétrole. Nous avons observé une relation dosage–réponse dans les taux respiratoires tels que mesurés par la consommation d'oxygène ($QO_2$). Nous avons observé des différences significatives ($P = .05$) dans les taux respiratoires des myes exposées à de faibles concentrations de pétrole. Les plus basses concentrations entraînent une augmentation du double des taux respiratoires et les hautes concentrations produisent un abaissement des taux. Les taux respiratoires des myes exposées à de faibles concentrations de pétrole diminuent à mesure que la teneur en hydrocarbures dans l'eau et dans le tissu des myes diminue, mais demeurent nettement différents de ceux des témoins. Les myes furent ensuite transférées dans un système non contaminé durant 14 jours après l'exposition au pétrole

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de 28 jours dans le but de déterminer les effets de l'épuration sur le taux respiratoire. Durant la période d'épuration, on constate que plusieurs des hydrocarbures présents dans le tissu des myes sont de nouveau des isomères naphtalène à méthyl substitué. Durant cette période, les taux respiratoires des myes initialement exposées à 10 ppm d'émulsion de fuel-oil demeurèrent nettement supérieurs à ceux des témoins. Les taux respiratoires de tous les groupes de myes exposées au pétrole se maintiennent à un taux différent de ceux des témoins, mais la différence a tendance à diminuer et les taux à se rapprocher de ceux des témoins. Il se peut qu'il y ait eu une réponse de narcose liée à la dose durant cette période.

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BIVALVE molluscs in the environment are frequently exposed to single spills or chronic discharges of petroleum oils. Chronic subdetectable oil pollution may occur from seeps, undetected spills, leaks, marine vessels, and urban and industrial sewage. Pollution incidents in temperate and subarctic estuarine and coastal marine waters will probably increase as further exploration, development, and transportation of oil increases. Lee et al. (1972), Stegeman and Teal (1973), and Neff and Anderson (1975) have reported the occurrence and uptake of petroleum hydrocarbons by bivalve molluscs. Various effects of petroleum oils and derivatives on bivalves have been described. Some of the effects reported were alterations of behavior (Galtsoff et al. 1935), and tissue structure (Barry and Yevich 1975; Stainken 1976a).

Few studies have characterized the effects of oils on the respiration of bivalves or related physiological responses to concentrations of contaminants in bivalve tissues. Gilfillian (1973, 1975) and Gilfillan et al. (1976) reported alterations in the carbon budgets of mussels and soft-shell clams exposed to crude and fuel oils and noted that $O_2$ consumption was altered. Avolizi and Nuwayhid (1974) found that exposure to crude oils decreased the $O_2$ consumption of mussels. In contrast, Fong (1976) described an increase in respiration rates of soft-shell clams exposed to crude oil. It is difficult to compare directly some studies because of the varying test parameters (i.e. salinities, temperatures, methods of oil addition, period of exposure, animal size, species, etc.). Therefore, this study was performed to determine the effect(s) of uptake and discharge of petroleum hydrocarbons on the $O_2$ consumption of the soft-shell clam, *Mya arenaria*. Effects on $O_2$ consumption were determined during a 28-d exposure to subacute concentrations of No. 2 fuel oil emulsions and a subsequent depuration period of 12 d. The effect on the clams was then related to the body burden of petroleum hydrocarbons and specific fractions.

*Mya arenaria* was chosen for study because these clams occur frequently in areas receiving acute and chronic oil exposures. Oil spills and their effects on *M. arenaria* have been documented by Thomas (1973), and the occurrence of oil in *M. arenaria* has been reported by Scarratt and Zitko (1972). Mechanisms by which *M. arenaria* may accumulate oil have been described by Stainken (1975) and Fong (1976). A No. 2 fuel oil was chosen for study because it is commonly used in coastal industrial installations and shipped in coastal waters. Experimentation was performed at 4°C because spills are more likely to occur during inclement winter weather.

The oils were added in an emulsified form to simulate a potential naturally occurring condition. Forrester (1971) and Gordon et al. (1973) reported the formation of oil emulsions in seawater. In the event of an oil spill during the colder months, it is probable that much of the oil would be dispersed and emulsified in the water column through turbulent wave action.

## Materials and Methods

The No. 2 fuel oil was supplied by the U.S. Environmental Protection Agency, Industrial Environmental Research Laboratory, Edison, N.J., (specific gravity, 2.40 centistokes; composition, 14% aromatics and 86% nonaromatics according to ASTM method No. D2549-68). Oil-in-water emulsions were prepared according to Gruenfeld and Frederick (1977).

Clams with a mean shell length of 25 mm (Newcombe 1936; Pfitzenmeyer 1965) were collected from Sequine Point, Staten Island, N.Y. Young clams were used because they tend to have greater respiration and filtration rates than those of older bivalves (Read 1962; Walne 1972). The clams were acclimated to the experimental conditions for 6 d before the emulsions were added.

An exposure period of 28 d to No. 2 fuel oil emulsions having concentrations of 10, 50, and 100 ppm was utilized. Four, 20-gal covered aquaria containing 60 L of filtered seawater per aquarium (salinity = 20‰) were used. The seawater was collected from Sandy Hook Bay and filtered through a coarse plankton net to remove macrodebris. One aquarium served as a control. Each of the remaining aquaria received a sufficient volume of a stock oil emulsion to attain an initial concentration of either 10, 50, or 100 ppm of emulsified oil. The time of addition was termed Time 0. The water was continuously aerated and the temperature maintained at 4°C. The photoperiod was approximately 16L:8D. Previous studies (D. M. Stainken unpublished data) have shown that *M. arenaria* establishes normal $O_2$ consumption patterns within these experimental parameters.

The hydrocarbon contents of the Sandy Hook Bay seawater and the experimental aquaria water were determined using a Freon extraction technique (Gruenfeld 1973). Before the experiments, the hydrocarbon content of the seawater was analyzed to ensure no external contamination. Each week, a 400-mL sample was removed from the same depth at the center of each tank. Concomittant studies of hydrocarbon accumulation and depuration of the clams (Stainken 1976b), and the effects (Stainken 1976a), were

performed. The method used for hydrocarbon analyses was described by Stainken (1976b).

Beginning at Time 0, the $O_2$ consumption of 18 clams from each of the groups, control and oil-exposed, was measured every 4 d for 28 d. At the end of this period, each experimental group of clams was rapidly removed to clean 20-gal aquarium containing seawater as described above. As clams were transferred, the valves were wiped clean to remove deposited pseudofeces, etc. The $O_2$ consumption of 18 clams from each of the groups, control and oil-exposed, was then measured every 4 d for 12 d. Samples for hydrocarbon analyses were obtained on days 7 and 14.

The procedure used for $O_2$ consumption measurements was modified from that of Kennedy and Mihursky (1972). A Scholander respirometer (The Mark Co., Brockton, Mass.) was used. Each respirometer vessel received 8 mL of natural seawater collected with the clams. This seawater had been stored at 4°C in the dark. The water in the respirometer vessels was prefiltered through a 1.4- and 0.8-filter (Millipore Corp., Bedford, Mass.) and aerated 1 h at 4°C prior to use. To absorb $CO_2$, 0.2 mL of 10% KOH was placed in a plexiglass boat with a piece of No. 4 Whatman paper. The valves of the clams were gently wiped dry before the animals were placed in the respirometer vessels. Animals were equilibrated 1½ h prior to measuring $O_2$ uptake. $O_2$ consumption was than measured for 1 h. Measurements were made in the morning or afternoon at the same time for each experimental group. Procedural blanks were run with each experiment. Shell length was measured at the end of each experiment and dry tissue weight (minus shell) was determined by drying the tissue to constant weight at 75–80°C for 48 h. The $O_2$ consumption data was then calculated as $\mu$L $O_2$/mg dry weight per hour or $QO_2$. This method has been used by Kennedy and Mihursky (1972) and Widdows (1973); it decreases much of the variability inherent in molluscan oxygen consumption rates.

Respirometric results were analyzed by linear regression. For regression analysis the procedures of Ostle (1963) and Draper and Smith (1966) were used. An analysis of variance and "in level" comparisons of the mean $QO_2$ measurements of oil-exposed and depurated clams were performed according to Campbell (1974). Results were considered significant at the $P = 0.05$ level.

## Results

The dissolved $O_2$ and pH remained optimal in all aquaria throughout the 28-d exposure period; bacterial contamination was not apparent. Control animals were reactive to tactile stimuli and appeared to be feeding. Very little mortality occurred at any of the oil concentrations employed. The actual mortalities in the oiled phase of the experiment were controls 0.0%, 10 ppm 0.5%, 50 ppm 2.0%, 100 ppm 3.0%.

In the 10-ppm aquaria, the oil odor disappeared by the 3rd wk and the clams appeared to react more slowly than the controls to tactile stimuli. The 50-ppm aquaria still had a faint odor of oil by the 3rd wk and the clams reacted much more slowly to tactile stimuli. In the 100-pm aquaria, there was still a faint odor of oil detectable after 3 wk, and the clams appeared narcotized and were relatively unreactive.

During the 14-d depuration period, the 10-ppm clams appeared to recover their tactile sensitivity to a level similar to that of the controls, but the 50- and 100-ppm clams continued to react sluggishly. Mortality increased during the depuration period among the 50- and 100-ppm clams. The actual mortality in the depuration was controls 0, 10 ppm 0.1%, 50 ppm 18.26%, 100 ppm 13.63%. The seawater before all tests and in the control tank during the tests did not have detectable hydrocarbon contents (0.2 ppm). Within the oiled tanks, some of the oil emulsion had broken within 2 h after addition. As indicated in Table 1, much of the oil emulsion and water-soluble fraction remained in the water column after 2 h.

TABLE 1. Hydrocarbon concentration (ppm) in the water column during the 28-d exposure period. The Time 0 sample measurement was made 2 h after addition of the emulsified oil.

| Aquaria | Time 0 | Wk 1 | Wk 2 | Wk 3 | Wk 4 |
|---|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 | 0 |
| 10 ppm | 6.0 | 0.8 | 0.4 | 0.3 | 0.3 |
| 50 ppm | 31.5 | 1.2 | 0.7 | 0.6 | 0 |
| 100 ppm | 55.3 | 1.5 | 1.5 | 0.4 | 0.3 |

One week later, the detectable hydrocarbon concentrations in the water column of the oiled aquaria were similar. Much of the oil appeared bound to mucus which adhered to the glass cooling coils and tank walls or formed floating organic flocculent conglomerates. The IR spectra also showed the emergence of an apparent aromatic band at 3030 $cm^{-1}$. The week 2 data were similar to those of week 1 and a peak emerged at ca 3000 $cm^{-1}$.

By week 3, the measurable hydrocarbon content had dropped further, and in the 10- and 50-ppm tank samples the possible aromatic peak became less apparent. The 100-ppm tank sample still had a strong peak at 3030 $cm^{-1}$. At the end of the 4th wk, the hydrocarbon content in the tanks had fallen to almost trace concentrations and peaks were becoming indistinguishable.

The mucus–oil complex formation may have aided in the dissipation of hydrocarbons from the water column. The mechanism of the binding of oil in mucus by *M. arenaria* has been documented earlier (Stainken 1975). Mass spectrometric analysis demonstrated that the mucus contained predominantly dimethylnaphthalenes and trimethylnaphthalenes with paraffins, mainly in the $C_{14}$ and $C_{15}$ regions.

During the depuration period, measurable amounts of hydrocarbons in the aquaria water were not detected. There was an emergence of a peak at 3000 $cm^{-1}$ during the 2nd week in the experimental tanks.

Figure 1 illustrates the calculated regression of $QO_2$ of clams exposed to oil over the 28-d period, and Table 2 contains the correlation coefficient, slope, y-intercept, upper and lower confidence intervals for the regression lines of Fig. 1. Table 3 contains the mean $QO_2$ and standard errors for the depuration period.

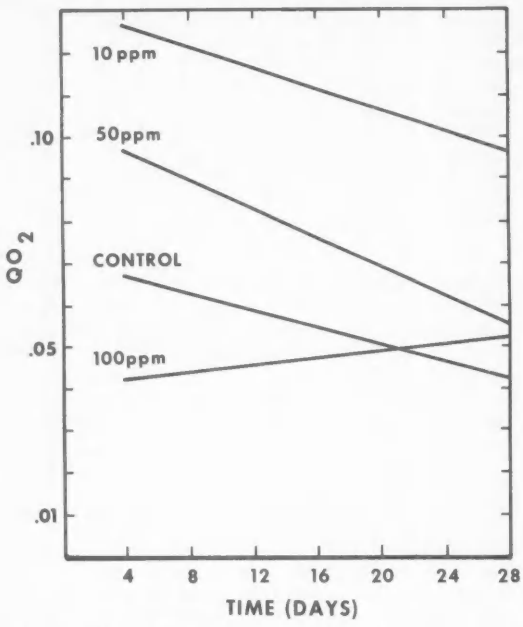


FIG. 1. Calculated regression of $QO_2$ of clams exposed to No. 2 fuel oil for 28 d.

The normal $QO_2$ rates of *M. arenaria* acclimated to 4°C ranged between 0.06 and 0.04 $QO_2$ units. This agrees with the results of Kennedy and Mihursky (1972) who found that clams acclimated and measured at 1°C had a $QO_2$ range from 0.07 to 0.40. Although these rates are not directly comparable due to temperature, latitude, and other unknown variables, it does indicate that $O_2$ consumption by *M. arenaria* is relatively low.

TABLE 3. Mean $QO_2$ and standard error (SE) or clams during the depuration period.

| | 4 d | 8 d | 12 d |
|---|---|---|---|
| Control | | | |
| $QO_2$ | .041 | .099 | .046 |
| SE | .008 | .016 | .008 |
| 10 ppm | | | |
| $QO_2$ | .065 | .154 | .087 |
| SE | .010 | .011 | .010 |
| 50 ppm | | | |
| $QO_2$ | .023 | .040 | .038 |
| SE | .007 | .011 | .008 |
| 100 ppm | | | |
| $QO_2$ | .043 | .120 | .058 |
| SE | .007 | .021 | .010 |

The 10-ppm regression of $QO_2$ in Fig. 1 appears to have translated to greater rates than those of the controls. After the initial exposure to oil and elevation of $QO_2$, there is a continual decline toward the control values. After 28 d, the 10-ppm $QO_2$ remained almost double the control values. During the depuration period, $O_2$ consumption by the 10-ppm-exposed clams remained elevated compared to the controls.

The 50-ppm regression of $QO_2$ in Fig. 1 was also elevated above that of the controls. Following an initial elevation, the 50-ppm rates gradually declined toward the levels of the controls. After 28 d of oil exposure, however, the 50-ppm rates remained elevated. During the depuration period, the rates were depressed slightly below those of the controls. This could be due to several factors including the more rapid loss of hydrocarbons possibly affecting cellular respiration, or generally depressed body condition subsequent to oil exposure.

The calculated regression of $QO_2$ of the 100-ppm

TABLE 2. Correlation coefficient ($r$), slope ($a_1$), y intercept ($a_0$), upper confidence intervals (UCI), and lower confidence intervals (LCI) for the regression lines of the control, 10 ppm, 50 ppm, and 100 ppm $QO_2$ rates of Fig. 1. *For each day measurement, $n = 18$.

| Control | 10 ppm | 50 ppm | 100 ppm |
|---|---|---|---|
| $a_0 = 0.072$ | $a_0 = 0.132$ | $a_0 = 0.103$ | $a_0 = 0.039$ |
| $a_1 = -0.001$ | $a_1 = -0.001$ | $a_1 = -0.002$ | $a_1 = 0.001$ |
| $r = -0.573$ | $r = -0.712$ | $r = -0.683$ | $r = 0.482$ |

| | 95% confidence intervals* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | | 10 ppm | | 50 ppm | | 100 ppm | |
| Day | UCI | LCI | UCI | LCI | UCI | LCI | UCI | LCI |
| 4 | .092 | .044 | .147 | .107 | .125 | .067 | .067 | .016 |
| 8 | .082 | .044 | .138 | .106 | .112 | .066 | .065 | .025 |
| 12 | .074 | .044 | .130 | .104 | .100 | .064 | .065 | .033 |
| 16 | .069 | .041 | .124 | .100 | .092 | .059 | .067 | .037 |
| 20 | .066 | .036 | .119 | .093 | .087 | .051 | .072 | .040 |
| 24 | .065 | .027 | .117 | .085 | .085 | .039 | .079 | .039 |
| 28 | .066 | .018 | .116 | .076 | .084 | .026 | .087 | .036 |

clams rotated around that of the controls (Fig. 1). Rates were depressed until the 16th d when the rates approximated those of the controls, followed by a continual elevation in rate until the end of the experiment. During the depuration period, oil consumption was slightly greater than the controls.

A single factor analysis of variance calculated for the mean $QO_2$ of the 28-d exposure period and the 12-d depuration period showed significance ($P = 0.001$) for the exposure period. Further "in level" analysis showed that differences existed between the control and 10-ppm rates ($P = 0.01$) and between those of the control and 50 ppm ($P = 0.05$). There was no significant difference between controls and 100 ppm. Comparisons of the depuration results were not significant ($P = 0.05$) for the rates of the controls and of those clams previously exposed to 50- and 100-ppm concentrations. However, the difference between the 10 ppm and controls was still significant ($P = 0.05$).

The total mean $QO_2$s for oil exposure and depuration periods were calculated. Generally the oil-exposed clams had greater $O_2$ consumption during exposure and depuration periods than the controls. The incidence of respiration was also increased by oil. The mean percent of clams respiring during the oil exposure period was controls 65%, 10 ppm 99.2%, 50 ppm 88%, and 100 ppm 75.4%. During depuration, the mean percent of clams respiring was controls 77%, 10 ppm 90.7%, 50 ppm 46.3%, and 100 ppm 72.2%.

## Discussion

The concentrations of oil in the water column began to decrease several hours after the addition of the oil emulsion. Several factors were probably responsible for this. Much of the oil was apparently removed from the water column by the mucociliary feeding and ejection mechanisms of the clams. The mechanisms for the formation of an oil–mucus complex and its chemical analysis were described earlier (Stainken 1975). A large content of oil, especially di- and trimethylnaphthalenes, was found in the mucus. Boehm and Quinn (1974) found that fuel oil could form droplets and micelles in seawater and that the major water soluble components were aromatic compounds, particularly naphthalenes.

After Time 0, the emulsified oil rapidly disappeared from the water column. The amount of dissolved compounds attained a peak measured in the 1st wk, and then decreased to equilibrium values (ranging from 1.5 to 0.3 ppm) for several weeks. An analysis of mucus and tissue from clams concomittantly exposed and sampled indicated that naphthalenes and methyl-substituted naphthalenes were probably predominant components in the water (Stainken 1976b). Anderson et al. (1974) have also reported that the water soluble fractions of oils often contains naphthalenes.

After 1-wk exposure, the peak accumulation of petroleum derived hydrocarbons was reached, followed by a gradual loss in accumulated hydrocarbons. Most of the materials accumulated were naphthalene and methyl substituted naphthalene isomers. Details of these chemical analyses were reported earlier (Stainken 1976b). Although other compounds were detected and accumulated, naphthalenes were the most persistent.

Depuration of accumulated hydrocarbons began as the hydrocarbon concentration in the water column decreased. When clams were placed in fresh seawater, depuration proceeded rapidly within the 1st wk, after which the rate slowed. Di- and trimethylnaphthalenes were still present in the clams after 2 wk in fresh seawater.

Gilfillan (1973) found that small amounts of oil extract could decrease the amount of energy available for maintenance, growth, and reproduction by decreasing the net carbon balance of the animal. Fong (1976) also found that the respiration rates of small *M. arenaria* were significantly increased after 3-wk exposure to seawater containing 220–370 g oil/L. In contrast, Dunning and Major (1974) reported that exposure to cold seawater extracts of No. 2 fuel oil tended to decrease $O_2$ consumption by mussels. Avolizi and Nuwayhid (1974) investigated the effects of a crude oil on two bivalve species and they also found a depression of respiratory rates at sublethal concentrations. Higher temperatures and physiological variation may possibly have influenced their results.

The results with *M. arenaria* in this study confirm that low levels of fuel oil cause a marked elevation in the respiratory rates. Higher concentrations cause a depression in the rates. As long as the oil- or water-soluble fraction is present, the rates remain affected. The actual content of hydrocarbons in the water column during the last 3 wk of the test ranged from 0.0 to 1.5 ppm. Even at the highest concentrations tested (100 ppm), as the concentrations of the water-soluble fraction decreased, the respiration rates increased. This apparently reflects a dose–response relationship. Very low sublethal concentrations of oil cause a doubling of the respiratory rates. Further support of a dose–response effect is evidenced in the accumulation of hydrocarbons. The greater uptake of hydrocarbons by clams exposed to 50 ppm may be due to a dose-dependent narcosis. Galtsoff et al. (1935) reported that water-soluble substances of crude oil could produce anesthetic effects on the ciliated epithelium of gills. The 100-ppm oil concentration may have reduced the filtration activity of the clams below that of the clams exposed to 50 ppm. The reduced filtration rate may account for the animals exposed to the higher concentration (100 ppm) accumulating less oil than the animals exposed to the lower concentration (50 ppm).

The mechanism(s) that cause the alterations in respiratory rates is unknown. Concomittant studies on the effects of oil exposure on tissue structure (Stainken 1976a) revealed a depletion of glycogen and generalized leukocytosis particularly evident in the blood sinuses of the pallium and mantle membrane. There was also

an increase in vacuolization of the diverticulum, stomach, and intestine. Effects appeared to be exacerbated at the higher exposure levels. Although the increase in respiratory rates is similar to an uncoupling effect of oxidative phosphorylation, further work on the cellular level is necessary. Altered $O_2$ consumption may be due to the disruption of normal cell membranal processes (Goldacre 1968) or cellular metabolism.


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# Absence of Aryl Hydrocarbon Hydroxylase (AHH) in Three Marine Bivalves[1,2]

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VANDERMEULEN, J. H., AND W. R. PENROSE. 1978. Absence of aryl hydrocarbon hydroxylase (AHH) activity in three marine bivalves. J. Fish. Res. Board Can. 35: 643–647.

Bivalves exposed to short-term (4 d) and long-term (6 yr) oil pollution were assayed for aryl hydrocarbon hydroxylase (AHH) and *N*-demethylase activity. Short-term induction studies were carried out on *Mya arenaria*, *Mytilus edulis*, and *Ostrea edulis* incubated in aqueous extracts of Kuwait crude oil or Bunker C (fuel) oil. For the chronic-induction studies *Mya arenaria* and *Mytilus edulis* were collected from oiled clam beds (*Arrow* Bunker C) in Chedabucto Bay, Nova Scotia. None of the bivalves showed any basal or petroleum-hydrocarbon-induced aryl hydrocarbon hydroxylase or *N*-demethylase activity, as shown by their inability to metabolize benzopyrene or imipramine. In contrast, oil-free control trout and trout taken from a polluted lake readily metabolized both these compounds. The inability of these bivalves to degrade petroleum aromatic hydrocarbons and the tendency of these compounds to accumulate in their tissues present an opportunity for transfer of unaltered hydrocarbons into the food chain.

*Key words:* aryl hydrocarbon hydroxylase, aromatic hydrocarbon, bivalve, *Mya arenaria*, *Mytilus edulis*, *Ostrea edulis*, petroleum, pollution

VANDERMEULEN, J. H., AND W. R. PENROSE. 1978. Absence of aryl hydrocarbon hydroxylase (AHH) activity in three marine bivalves. J. Fish. Res. Board Can. 35: 643–647.

Des analyses ont été effectuées sur des bivalves exposés durant une courte (4 jours) et une longue période (6 ans) à la pollution par le pétrole, afin de mesurer l'activité de l'aryl hydrocarbure hydroxylase (AHH) et de la N-déméthylase. Nous avons fait des études d'induction enzymatique à court terme sur *Mya arenaria*, *Mytilus edulis* et *Ostrea edulis* incubés dans des extraits aqueux de pétrole brut de Koweït ou de fuel de soute C. Pour les études d'induction chronique, *Mya arenaria* et *Mytilus edulis* furent *prélevés* sur des bancs de coques affectés par le pétrole (fuel de soute C de l'*Arrow*) dans la baie Chédabouctou, Nouvelle-Ecosse. Aucun des bivalves ne montra d'activité des deux enzymes déclenchée par les hydrocarbures de base ou de pétrole, tel que démontré par leur incapacité à métaboliser le benzopyrène ou l'imipramine. Par contraste, des truites témoins non touchées par le pétrole et des truites provenant d'un lac pollué métabolisèrent facilement ces deux composés. Par suite de leur incapacité à dégrader les hydrocarbures aromatiques du pétrole et de la tendance de ces composés à s'accumuler dans les tissus, ces bivalves permettent le transfert d'hydrocarbures inaltérés dans la chaîne alimentaire.

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THERE is doubt that molluscs can metabolize petroleum hydrocarbons. Lee and co-workers did not find metabolites of naphthalene, toluene, and 3,4-benzopyrene in mussels incubated in seawater containing these compounds (Lee et al. 1972). Similarly, Payne and Penrose (1975b) were unable to detect either basal or petroleum-induced levels of aryl hydrocarbon hydroxylase (AHH) in the intertidal snail *Littorina* sp. incubated in the laboratory in oiled seawater. In higher animals metabolism of aromatic petroleum hydrocarbons is handled by the liver's AHH system (Orrenius and Ernster 1974). The inducibility of this system by various polycyclic hydrocarbons has been demonstrated (e.g. Pedersen et al. 1976), and environmental induction of the AHH

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[2]Bedford Institute of Oceanography Contribution.

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system in the field has been shown for several fish taken from variously polluted waters (Payne and Penrose 1975a; Payne 1976; Burns 1976).

The AHH system forms an important part of the environmental biological response to oil contamination, and the possible absence of this mechanism in one or more major components of a food chain becomes significant in considering the recovery potential of a community after a spill. For this reason we analyzed for AHH activity clams and mussels from chronically oiled beds in Chedabucto Bay, N.S., the site of the *Arrow* spill in 1970. Results are discussed in terms of hydrocarbon accumulation in the food chain.

## Materials and Methods

Oil-free soft-shell clams (*Mya arenaria*) and blue mussels (*Mytilus edulis*) were collected from the eastern shore, 50 km north of Halifax, N.S. Oil-free European oysters (*Ostrea edulis*) were obtained from the Nova Scotia Department of Fisheries oyster culture station, also on the eastern shore, an area free of petroleum hydrocarbon contamination. Chronically oiled (6 yr) *Mya arenaria* and *Mytilus edulis* were collected from Chedabucto Bay, N.S., at Moussiliers Passage and Petit Barachois, areas heavily oiled with Bunker C fuel oil after the tanker *Arrow* sank in February 1970 (Anon. 1970). High oil concentrations remain in and on the sediments (Vandermeulen and Gordon 1976). Only bivalves 6 yr and older were taken for study. Ages were determined by inspection of shell growth annuli.

Chronically oiled *Mya arenaria* were maintained in glass aquaria in oil-free seawater and depuration was determined by fluorescence spectroscopy on tissue hexane extracts. Results were expressed as "Bunker C equivalents" by comparison with a standard prepared from original *Arrow* Bunker C oil. Bunker C oil in bivalve tissues was identified by two-dimensional fluorescence intensity contour plots (Hargrave and Phillips 1975).

For AHH induction studies the molluscs were exposed for 4 d to Kuwait crude oil or Bunker C fuel oil (Gulf Oil Canada Ltd.). The animals were kept in glass aquaria in 10 L of aerated seawater to which an aqueous oil extract had been added. The aqueous extract was prepared by shaking 5 mL of either Kuwait crude or Bunker C in 250 mL seawater in glass jars on a Burrell wrist-action shaker (15 min, setting 10). After phase separation in a separatory funnel for 1 min, 200 mL of the lower aqueous phase was added directly to the seawater in the aquaria. A further 200 mL of aqueous oil extract was again added on day 3 of the 4-d incubation period. Prepared in this manner hydrocarbon concentrations in the aquaria ranged from 4.3 to 24.9 ppm (mg/L) for Kuwait crude and from 2.0 to 1.7 ppm (mg/L) for Bunker C during the induction period.

AHH activity was assayed by monitoring hydroxylation of benzopyrene to 3-OH-benzopyrene (Nebert and Gelboin 1968). The appearance of the metabolite was followed by measurement of alkali-extractable fluorescence (Perkin-Elmer fluorescence spectrophotometer, MPF-2A). An alternative route of aromatic hydrocarbon degradation, *N*-demethylation, was assayed by monitoring demethylation of imipramine. Imipramine-$^{14}$C (1 $\mu$Ci; Amersham-Searle) was substituted for benzopyrene in the Nebert and Gelboin assay, and the metabolites extracted and separated by the method of Bickel and Weder (1968). $^{14}$C-imipramine metabolites were determined by scanning thin-layer chromatography (Packard radiochromatogram scanner, Model 7201). Incubations (at 25°C) were done with whole bivalve homogenates and with all cofactor requirements fulfilled as per Nebert and Gelboin (1968).

Oil-free yearling brook trout (*Salvelinus fontinalis*) served as controls for the benzopyrene assay. AHH induction in trout was carried out as for the molluscs. Brown trout (*Salmo trutta*) with high AHH levels taken from a polluted lake near St. John's, Nfld., served as controls for the imipramine assay.

TABLE 1. Distribution of hydrocarbons in tissues ($\mu$g/g net weight) of chronically oiled *Mya arenaria* after 92-d depuration in oil-free seawater. Excised tissues of 10 specimens were pooled.[a]

| Tissue | Tissue hydrocarbon concn ($\mu$g/g net wt) | |
|---|---|---|
| | 3 d | 92 d |
| Mantle | 14.3 | 0 |
| Mantle edge | 16.1 | 7.9 |
| Siphon | 11.2 | 4.5 |
| Siphon epidermis | 92.8 | 22.0 |
| Gills and lappets | 0 | 3.5 |
| Gut/hepatopancreas/foot | 9.1 | 11.4 |
| Style | 0 | 0 |
| Adductor muscle | 7.6 | 8.4 |
| Hinge ligament | 0 | 0 |

[a]Determined on hexane extracts (Vandermeulen and Gordon 1976) by comparison with *Arrow* Bunker C known standard concentrations. All values corrected for normal fluorescence background levels.

## Results

### Depuration

Clams from chronically oiled Chedabucto beaches contained high tissue levels of petroleum hydrocarbons. After transfer to oil-free seawater these clams lost hydrocarbons rapidly during the first 24–48 h, and gradually thereafter. However, even after 75-d depuration tissues still retained up to 40% of the initial hydrocarbon load, still identifiable as petroleum derived (Fig. 1). Even after nearly 3-mo depuration measurable amounts of Bunker C-derived hydrocarbons still remained in various tissues of *Mya arenaria*.

The most highly contaminated tissues at the beginning of a 92-d experiment (Table 1), the siphon epidermis, mantle, gills, and lappets were also the tissues in which depuration occurred most markedly. Interestingly, hydrocarbon levels remained fairly constant in the gut–hepatopancreas–foot complex and in the adductor muscles.

### Aryl Hydrocarbon Hydroxylase (AHH) Activity

Non-oiled hatchery trout maintained in oil-free water possessed a low basal level of AHH activity (Table 2). This was markedly enhanced by exposure for 4 d to

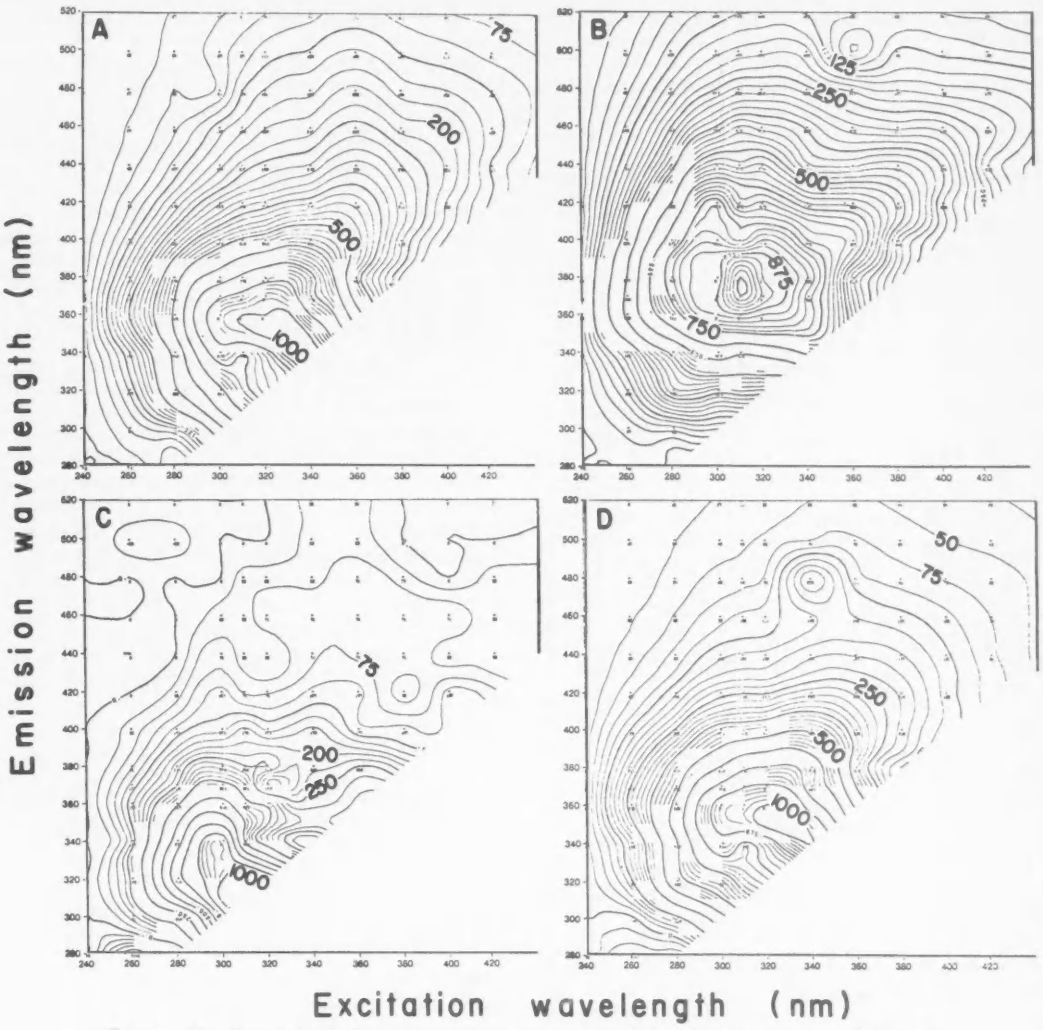


FIG. 1. Two-dimensional fluorescence intensity contour plots of A, *Mya arenaria* from non-oiled sediments; B, stranded Bunker C from an oiled clam bed at Chedabucto Bay; C, *M. arenaria* from an oiled clam bed; and D, oiled *M. arenaria* after 75-d depuration in oil-free seawater.

aqueous extracts of either Kuwait crude or Bunker C fuel oil. Although the results are highly variable, as shown by the size of the standard error, enhancement was generally greater in Bunker C-treated trout than in those exposed to Kuwait crude oil. In contrast, none of the bivalves tested showed any detectable benzopyrene hydroxylation, including the chronically oiled Chedabucto Bay clams and mussels. However, all test organisms contained detectable petroleum hydrocarbons in their tissues at the time of the AHH assay.

Radiochromatograms of trout liver homogenate incubated with $^{14}$C-imipramine showed the imipramine peak as well as its two metabolite peaks, indicative of AHH activity (Table 3). No metabolite peaks could be detected in preparations of bivalve homogenates incubated with imipramine.

## Discussion

This study shows mainly that in the field these bivalves clearly lack the enzyme mechanism to deal with exogenous aromatic hydrocarbons. This supports earlier laboratory studies (Payne and Penrose 1975b).

The initial rapid drop in tissue fluorescent hydrocarbons during the first 48 h of depuration is probably due to loss of hydrocarbons adhering to exposed epi-

TABLE 2. Aryl hydrocarbon hydroxylase activity, as determined by benzopyrene hydroxylation, in non-oiled and oiled bivalve molluscs and brook trout. ($N = 5$ for all assays).

| Test organism | Treatment | AHH activity $\bar{x} = SE$ |
|---|---|---|
| *Salvelinus fontinalis* | Control | 0.23 ± 0.23 |
| | Kuwait crude | 21.50 ± 8.06 |
| | Bunker C | 54.28 ± 41.37 |
| *Mya arenaria* | Control | 0 |
| | Kuwait crude | 0 |
| | Bunker C | 0 |
| | Chedabucto Bay | 0 |
| *Mytilus edulis* | Control | 0 |
| | Chedabucto Bay | 0 |
| *Ostrea edulis* | Control | 0 |
| | Kuwait crude | 0 |
| | Bunker C | 0 |

TABLE 3. Imipramine demethylation in *Mya arenaria* and *Mytilus edulis* from non-oiled and chronically oiled sediments and in brown trout from a polluted lake.

| Test organism | Source | Oil concn (μg/g protein N) | AHH activity |
|---|---|---|---|
| *Salmo trutta* | Quidi Vidi Lake, Nfld. | — | + |
| *Mya arenaria* | Eastern shore, N.S. | 0 | 0 |
| | Chedabucto Bay, N.S. | 14.21 ± 6.02 | 0 |
| *Mytilus edulis* | Eastern shore, N.S. | 0 | 0 |
| | Chedabucto Bay, N.S. | 0 | 0 |

[a]Indicated by presence (+) or complete absence (0) or $^{14}$C-imipramine metabolite peaks on thin-layer chromatograms.

thelial surfaces such as the mantel and gills and the excretion of oiled debris already in the gut at the time of collection. The more interesting part of the depuration curve, however, is the gradual decline in the tissue-bound hydrocarbons thereafter and the long residence time within certain tissues, especially the gonads. This raises the environmental concern that these accumulated petroleum aromatic hydrocarbons, held to be more toxic or deleterious than the straight- or branched-chain aliphatics (Morrow et al. 1975; Corner 1975), appear to be especially long lived in those tissues that contain the genetic information for the next generations. The precise sites of these accumulated aromatic hydrocarbons is not known, but presumably includes the lipid stores of the tissues because of their lipophilic nature. The relationship between mollusc lipid stores and hydrocarbon persistence in the tissues of chronically oiled organisms needs to be determined, particularly in relation to embryogeny. Nothing is known of the hydrocarbon effects during this critical phase in the life cycle or of the possible transfer of aromatics from adult to embryo.

Our finding that these bivalves lack AHH enhancement in response to petroleum hydrocarbon confirms the tentative suggestion of Lee et al. (1972) and Payne (1976). However, a cytochrome ($P_{450}$ or $P_{448}$) based AHH system does apparently exist in some molluscs. Khan et al. (1972) showed the epoxidation of aldrin in the bivalve mollusc *Anondonta* sp. and the gastropod *Lymnaea palustris*, which implies the existence of such a system. Thus the induction of the cytochrome $P_{450}$-based mixed function oxidase system in bivalves is probably substrate specific (for reviews see Orrenius and Ernster 1974, p. 229; Jerina and Daly 1974, p. 575; Parke 1975, p. 209) and is not initiated by the hydrocarbons we studied. The discussion is academic, however, when seen in terms of the metabolic requirements or response potential of these molluscs in the event of either short-term or chronic oil exposure. Whatever the situation in the laboratory, under field conditions the normal AHH mechanism cannot be brought into play in these molluscs, and in the absence of other mechanisms such as hydroxylating peroxidases or phenol conjugation, the only defense appears to be exceedingly slow depuration.

The ecological consequences of slow and incomplete depuration of oiled clams are twofold. First, utilization as human food is delayed; second, molluscs often make up a significant percentage of benthic fauna and contribute significantly to the total fixation turnover of sediment. The apparent inability of these molluscs to degrade polycyclic aromatic hydrocarbons and the tendency of these compounds to accumulate in their tissues provide an opportunity where unaltered aromatic hydrocarbons may enter the food chain.

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# Long-Term Effects of an Oil Spill on Populations of the Salt-Marsh Crab *Uca pugnax*[1,2]

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KREBS, C. T., AND K. A. BURNS. 1978. Long-term effects of an oil spill on populations of the salt-marsh crab *Uca pugnax*. J. Fish. Res. Board Can. 35: 648–649.

A spill of fuel oil at West Falmouth, Massachusetts, in 1969 contaminated contiguous salt marshes with up to 6000 μg oil/g (ppm) of wet mud and affected local populations of the salt-marsh crab *Uca pugnax*. Directly related to high sediment oil content were reduced crab density, reduced ratio of females to males, reduced juvenile settlement, heavy overwinter mortality, incorporation of oil into body tissues, behavioral disorders such as locomotor impairment, and abnormal burrow construction. Concentrations of weathered fuel oil >1000 ppm were directly toxic to adults, while those of 100–200 ppm were toxic to juveniles. Cumulative effects occurred at lower concentrations. Recovery of the marsh from this relatively small oil spill is still incomplete after 7 yr. Interpretation of the crab population data upon the basis of detailed analysis of the aromatic fraction of the fuel oil provided by Dr J. Teal, Woods Hole Oceanographic Institution, showed that recovery of the crab populations was highly correlated with the disappearance of the naphthalene fraction of the aromatics. There had been a decrease in the aromatics from 42% in 1970 to approximately 18% in 1976 in Station I surface sediments, while preliminary analyses show aromatics still high at other stations where little recovery has been observed. By 1970–71 all parent naphthalene compounds were gone in Station I surface sediments, but substituted naphthalenes were still in high concentrations. By 1972–73 dimethyl naphthalenes and $C_3$ and $C_1$ substituted naphthalenes remained at about 25% of original values. By 1976–77 only $C_3$ and $C_4$ substituted naphthalenes remained at less than one tenth of their 1973 levels. In 1972–73 the substituted naphthalenes were at high enough concentrations to prevent recruitment, as large juvenile crab settlements in 1970–73 produced no recruitment into the crab populations. By 1976–77 these toxic compounds were at low enough levels that recovery of the crab population was occurring with recruitment, and increasing density was observed in both years. High aromatic concentrations at other stations may still be inhibiting recovery at these stations. The long-term inhibition of recruitment and low population densities may have resulted from exposure to oil in the interstitial waters during the sensitive molt period and/or during the long periods of time while the crabs were overwintering in the substrate.

*Key words:* petroleum, *Uca pugnax*, crab, salt-marsh, population, pollution

KREBS, C. T., AND K. A. BURNS. 1978. Long-term effects of an oil spill on populations of the salt-marsh crab *Uca pugnax*. J. Fish. Res. Board Can. 35: 648–649.

Un déversement de fuel-oil se produisit à West Falmouth, Massachusetts, en 1969, contaminant les marais salés avoisinants avec des quantités de pétrole allant jusqu'à 6 000 μg de pétrole/g (ppm) de vase humide et affectant les populations locales de *Uca pugnax*. Les effets montrant une relation directe avec une haute teneur en pétrole dans le sédiment sont les suivants : densité réduite des crabes, nombre moindre de femelles par rapport aux mâles, fixation d'un nombre moindre de jeunes, sérieuses mortalités d'hiver, incorporation du pétrole aux tissus corporels, désordres éthologiques tel dérangement de la locomotion, et construction anormale des tunnels. Des concentrations de fuel-oil dégradé

[1]This paper forms part of the Proceedings of the Symposium on "Recovery Potential of Oiled Marine Northern Environments" held at Halifax, Nova Scotia, October 10–14, 1977.

[2]The text of this contribution to the Symposium, and a portion of the abstract, has been previously published in *Science* 197: 484–487. The text is not repeated here.

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dépassant 1 000 ppm sont directement toxiques pour les adultes, alors que celles de 100 à 200 ppm sont toxiques pour les jeunes. Il se produit des effets cumulatifs à de plus faibles concentrations. Après 7 ans, les marais ne se sont pas encore rétablis de ce déversement de pétrole relativement faible. Nous avons interprété les données relatives aux populations de crabes en nous basant sur l'analyse détaillée de la fraction aromatique du fuel-oil que nous a fourni M. John Teal, de la Woods Hole Oceanographic Institution. On constate que le rétablissement des populations de crabes est en étroite corrélation avec la disparition de la fraction naphtalène des composés aromatiques. Il y eut diminution des composés aromatiques depuis 42% en 1970 à environ 18% en 1976 dans les sédiments de surface de la station I, alors que des analyses préliminaires démontrent des niveaux de substances aromatiques encore élevés aux autres stations où nous n'avons observé que peu de rétablissement. En 1970–71, tous les composés apparentés à la paphtalène étaient disparus des sédiments de surface de la station I, mais les concentrations de naphtalènes substituées étaient encore élevées. En 1972–73, les diméthylnaphtalènes et les naphtalènes substituées $C_3$ et $C_4$ se maintenaient à environ 25% des valeurs originelles. En 1976–77, seules les naphtalènes substituées $C_3$ et $C_4$ demeuraient à moins de un dixième de leurs niveaux de 1973. En 1972–73, les naphtalènes substituées étaient présentes en concentrations suffisamment élevées pour empêcher le recrutement, car les abondantes fixations de jeunes crabes en 1970–73 ne produisirent aucun recrutement des populations de crabes. En 1976–77, ces composés toxiques se trouvaient à des niveaux suffisamment bas pour permettre à la population de crabes de se rétablir, un recrutement et une augmentation de densité étant observés durant ces deux années. De fortes concentrations de substances aromatiques aux autres stations peuvent encore empêcher le rétablissement à ces stations. Il se peut que l'inhibition à long terme du recrutement et les faibles densités de population aient résulté de l'exposition au pétrole dans les eaux interstitielles durant la délicate période de mue et/ou les longues périodes d'hivernement des crabes dans le substrat.

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# Effects of Chronic Exposure to Petroleum Upon the Growth and Molting of Juveniles of the Arctic Marine Isopod Crustacean *Mesidotea entomon*[1]

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PERCY, J. A. 1978. Effects of chronic exposure to petroleum upon the growth and molting of juveniles of the arctic marine isopod crustacean *Mesidotea entomon*. J. Fish. Res. Board Can. 35: 650–656.

Juveniles of the benthic marine isopod *Mesidotea entomon* were chronically exposed to different concentrations of water-soluble fractions of fresh and weathered Norman Wells crude oil and of fresh Pembina crude for 160 d. The 100% extracts contained 1.72, 1.12, and 0.56 ppm of oil (determined fluorimetrically), respectively. Most of the animals completed five or six molts before the end of the experiment. Long-term mortality was high in the 100% extracts of the oils (LT50 = 17, 17, and 41 d, for Norman Wells, Pembina, and weathered Norman Wells, respectively) but most of the animals molted at least once before dying. None of the deaths occurred in conjunction with the molt. Stimulation of the onset of the subsequent molt occurred in some exposure groups. A significant increase in the duration of the intermolt period only occurred at the highest oil concentrations. Effects on growth were slight at concentrations lower than that which is lethal during chronic exposure. Exposure to fresh Norman Wells crude depressed growth slightly, while Pembina crude slightly stimulated growth. Weathered Norman Wells severely inhibited growth at the highest concentration but stimulated growth slightly at lower concentrations.

*Key words:* petroleum, growth, molting, arctic, isopod, *Mesidotea entomon*

PERCY, J. A. 1978. Effects of chronic exposure to petroleum upon the growth and molting of juveniles of the arctic marine isopod crustacean *Mesidotea entomon*. J. Fish. Res. Board Can. 35: 650–656.

Nous avons soumis des jeunes de l'isopode marin benthique *Mesidotea entomon* à une exposition chronique à différentes concentrations de fractions solubles dans l'eau de pétrole brut de Norman Wells frais et altéré à l'air, et de pétrole brut frais de Pembina pendant 160 jours. Les extraits à 100% contiennent 1.72, 1.12 et 0.56 ppm de pétrole (déterminé par fluorométrie), respectivement. La plupart des animaux complétèrent cinq ou six mues avant la fin de l'expérience. La mortalité à long terme est élevée dans les extraits à 100% des pétroles ($TL_{50}$ = 17, 17 et 41 jours, pour le pétrole frais de Norman Wells et de Pembina, et le pétrole altéré de Norman Wells, respectivement), mais la plupart des animaux muèrent au moins une fois avant de mourir. Aucune mortalité ne se produisit en rapport avec la mue. Dans quelques groupes exposés, il se produisit une stimulation déclenchant une mue subséquente. Ce n'est qu'aux plus hautes teneurs en pétrole qu'il y eut augmentation significative de la durée de la période d'intermue. Les effets sur la croissance sont faibles à des concentrations inférieures à celle qui est létale lors de l'exposition chronique. L'exposition à du pétrole brut frais de Norman Wells ralentit légèrement la croissance, alors que le pétrole brut de Pembina la stimule. Le pétrole altéré de Norman Wells ralentit sérieusement la croissance à la concentration la plus élevée, mais la stimule légèrement aux faibles concentrations.

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LITTLE is known about the effects of chronic exposure of arctic marine organisms to low concentrations of petroleum hydrocarbons. In this study the effects of exposure to water-soluble fractions (WSF's) of fresh and weathered crude oil upon survival, growth, and molting of young of the isopod *Mesidotea entomon* were examined.

Three isopods of the genus *Mesidotea* (*M. entomon*, *M. sibirica*, and *M. sabini*) are abundant in the southern Beaufort Sea. They are an important component of the benthic community, acting as voracious scavengers and serving as food for a variety of fish and marine mammals. Their general biology and distribution in this area

[1]This paper forms part of the Proceedings of the Symposium on "Recovery Potential of Oiled Marine Northern Environments" held at Halifax, Nova Scotia, October 10–14, 1977.

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have been considered by Bray (1962). Current work at the Arctic Biological Station shows that *M. entomon* penetrates farthest into the shallow coastal areas that are most strongly influenced by the warm, fresh outflow of the Mackenzie River. Furthermore, gravid females are found only in the very shallow, brackish warm water. Bray (1962) had found that very small juveniles are abundant close to shore but are absent in deeper water. The shoreline and shallow benthic habitat where the juveniles are concentrated is particularly vulnerable to contamination by spilled oil.

Adult isopods are very resistant to crude oil (Percy and Mullin 1975; Percy 1976, 1977). However, nothing is known about the sensitivity of the newly released young. Studies demonstrating that larvae and young of many species are more readily killed by petroleum than are adults have been reviewed by Moore and Dwyer (1974) and by Rice et al. (1977).

Rapid mortality is not the only ecologically significant consequence of oil pollution. Crude oil can disrupt many biological processes without causing immediate death. Anderson (1977) has reviewed some of the more important of these sublethal effects. Changes in the molt process and cycle have been used as sensitive indicators of environmental stress (Epifanio 1971). Growth is also a useful indicator because disturbance of this process generally entails clear ecological consequences and probably reflects the cumulative impact of several interacting physiological, biochemical, or behavioral dysfunctions.

## Materials and Methods

### Source of Animals

Gravid *Mesidotea entomon* were collected in August in baited traps in Ptarmigan Cove, Herschel Island, N.W.T. (69°34′N, 138°59′W) and transported to the Arctic Biological Station in seawater. They were maintained in recirculating seawater (Instant Ocean, 15°C, 15‰) until young were released (300–500 young per female). The mean body length of the young at the time of release was $4.01 \pm 0.18$ mm. The young from several females were placed in a single tank and groups of 3–4-d-old animals selected at random for use in the growth–molting study.

### Preparation of Oil–Water Mixtures

Three northern oils were used in the study: fresh Norman Wells crude (NW); weathered Norman Wells crude (WNW) prepared by stirring 100 mL samples of oil in shallow, open, glass dishes at 5°C under a constant airflow for 72 h, which results in a weight loss of approximately 34%; and fresh Pembina crude (P). A description of the physical characteristics of these northern oils is presented by Keevil and Ramseier (1975).

Water-soluble fractions (WSF's) were prepared by placing 20 mL of oil in an aspirator bottle with 2.1 L of aged, synthetic seawater (Instant Ocean, 15‰, 5°C). The bottles were loosely covered with aluminum foil and stirred slowly at 5°C with a magnetic stirrer for 20 h. This solution was left undisturbed for 30 min and then 1700 mL was drawn off from under the oil layer and thoroughly mixed. This 100% WSF was then used to prepare 50 and 10% dilutions. Subsamples (100 mL) were collected for fluorescence analysis. The concentration of hydrocarbons was measured in duplicate samples of freshly prepared test solutions ("initial") and in samples obtained by pooling old medium from several exposure jars at the time of replacement with fresh medium ("final"). Values for the "initial" samples thus represent the maximum concentration of hydrocarbons to which the animals were exposed at the commencement of each period in fresh medium, while the "final" values represent the minimum concentration of hydrocarbons at the time of the subsequent water change. The quantity of oil dissolved in the seawater was estimated spectrofluorimetrically by the method of Keizer and Gordon (1973). The test solutions were warmed to 10°C before being added to the animal holding vessels.

### Exposure of Animals

Twenty animals were removed at random from the stock tank for determination of initial body length and dry weight. Similar groups of 20 animals were placed in each test solution. Three oils and three concentrations of each (100, 50, and 10% of stock solution) required a total of 180 animals. An additional three groups of 20 animals each were selected as controls (CON A, CON B, CON C). The animals were held individually in 60-mL glass bottles, with perforated screw caps, containing 45 mL of the test solution. The bottles were kept at $10 \pm 0.2$°C and the media replaced every third day. Animals were fed an excess ration of tetramin fish food. At 2–3-wk intervals the animals were transferred to clean bottles.

Dead animals and molted exoskeletons were removed and recorded daily. Ecdysis occurs in two parts, the tail first, followed by the cephalothorax, usually within 24 h. The time of ecdysis was determined as the first appearance of the molted tail section. The duration of each successive intermolt period was determined for each individual in each exposure series.

Body lengths (from the rostrum to the tip of the telson) of all the animals were measured after 84 d with a dissecting microscope equipped with a calibrated ocular micrometer. Lengths of animals in the initial sample and in all groups at the end of the experiment (after 160 d of growth) were measured with a vertical projector of the type designed for reading fish scales. Animals in the initial sample and all those remaining at the end of the experiment were dried to constant weight at 70°C and weighed individually.

The significance of the differences in mean values of intermolt duration, body length, and body weight between the combined control groups and each exposure group was determined by Student's *t*-test.

## Results

### Concentration of Hydrocarbons in the WSF

The measured concentrations of petroleum hydrocarbons to which the animals were exposed are presented in Table 1. The 100% seawater extract of fresh Norman Wells crude had the highest initial hydrocarbon concentration (1.71 ppm). The weathered oil yielded a WSF containing about 40% less hydrocarbon (1.01 ppm) than did the extract of fresh oil. The hydrocarbon concentration in the WSF of Pembina crude (0.50

TABLE 1. Maximum (initial) and minimum (final) concentrations of hydrocarbons in the seawater extracts of Norman Wells (NW), weathered Norman Wells (WNW), and Pembina (P) crude oils to which the juvenile *M. entomon* were exposed. A. Mean initial and final concentrations for first 10 water changes. B. Mean initial concentration for duration of study. Δ% = percent decline in hydrocarbon concentration between water changes.

| Oil | % of stock | Initial concn | | | | Final concn | | | | Δ% |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *N* | $\bar{x}$(ppm) | SD | SE | *N* | $\bar{x}$(ppm) | SD | SE | |
| **A.** | | | | | | | | | | |
| NW | 100 | 9 | 1.71 | 0.17 | 0.06 | 6 | 1.00 | 0.12 | 0.05 | −41.5 |
| | 50 | 10 | 0.90 | 0.13 | 0.04 | 10 | 0.50 | 0.11 | 0.03 | −44.4 |
| | 10 | 10 | 0.19 | 0.06 | 0.02 | 10 | 0.11 | 0.07 | 0.03 | −42.1 |
| WNW | 100 | 10 | 1.01 | 0.17 | 0.06 | 10 | 0.56 | 0.18 | 0.06 | −44.6 |
| | 50 | 10 | 0.52 | 0.12 | 0.04 | 10 | 0.28 | 0.07 | 0.03 | −46.2 |
| | 10 | 10 | 0.10 | 0.02 | 0.01 | 10 | 0.09 | 0.06 | 0.02 | −10.0 |
| P | 100 | 10 | 0.50 | 0.12 | 0.04 | 10 | 0.24 | 0.05 | 0.02 | −52.0 |
| | 50 | 10 | 0.26 | 0.10 | 0.03 | 10 | 0.09 | 0.03 | 0.01 | −65.4 |
| | 10 | 10 | 0.04 | 0.02 | 0.01 | 10 | 0.02 | 0.03 | 0.01 | −50.0 |
| **B.** | | | | | | | | | | |
| NW | 100 | 25 | 1.72 | 0.30 | 0.04 | | | | | |
| WNW | 100 | 24 | 1.12 | 0.20 | 0.04 | | | | | |
| P | 100 | 21 | 0.56 | 0.13 | 0.03 | | | | | |

ppm) was less than a third of that in the WSF of fresh NW crude. The concentration proportions were essentially similar in the diluted extracts.

The hydrocarbon concentrations in the exposure jars declined considerably during the 3 d between water changes (Table 1). Slightly more than half of the soluble material was lost from the WSF of fresh and weathered NW crude, while slightly less than half was lost from the extracts of Pembina crude.

### LETHAL EFFECTS

Each of the oils differed in toxicity, although the 100% extract of all three exceeded the chronic lethal level for the isopods (Fig. 1). Pembina crude was more toxic than NW crude. Mortalities among animals exposed to the 100% extracts of fresh NW (1.71 ppm) and Pembina (0.50 ppm) crudes were similar. In both, 50% of the animals died within 17 d, and all were dead by 30 and 37 d, respectively. The 100% WSF of the weathered NW crude containing 1.01 ppm hydrocarbons caused fewer deaths than did the fresh oil; 50% mortality occurred after 41 d of exposure, and 30% of the animals were still alive at the conclusion of the study.

Neither the 50% nor the 10% extracts of any of the oils resulted in 50% mortality prior to the conclusion of the experiment. Twenty-five percent of the animals exposed to the 50% WSF of fresh NW crude (0.90 ppm) died before the end of the experiment. Mortalities in all of the other exposure groups were <15% and were comparable to the values for control animals.

### EFFECTS ON MOLT CYCLE

In the 100% WSF of all three oils most of the animals molted at least once before dying. Only in fresh NW and P extracts did significant numbers of animals die without molting (25 and 15%, respectively). Only in the 100% WSF of weathered NW did a significant number of animals (35%) molt more than once before dying. Of those animals that died after molting once, none died during ecdysis or within a short period after it. In the 100% extract of fresh NW and P crudes the mean time between ecdysis and death was similar (14.7 ± 4.8 d and 14.5 ± 7.7 d, respectively). In contrast, for animals exposed to the 100% extract of weathered NW crude the mean time between the first molt and death was 35.2 ± 26.6 d (considering just those animals that molted only once before dying). This period is far longer than the normal intermolt period for animals of this age (23.6 ± 2.4 d).

### EFFECT ON ONSET OF ECDYSIS

For the three control groups the time after the beginning of the experiment for 50% of the animals to molt ranged from 5.5 to 7.5 d. Comparable times were required for animals exposed to all three concentrations of fresh NW crude. In contrast, an acceleration in the onset of ecdysis occurred in animals exposed to 100 and 50% WSF's of weathered NW and P crudes; the time required for 50% of the animals to molt ranged from 2.5 to 4.0 d. The onset of ecdysis in animals exposed to the 10% extract of both these oils was not accelerated relative to the controls.

### EFFECT ON INTERMOLT DURATION

The lengths of five successive intermolt periods were determined for most of the exposure groups (Table 2). The mean period for the control animals progressively increased from about 26 d for the first period measured to 45 d for the fifth period. In assessing the significance

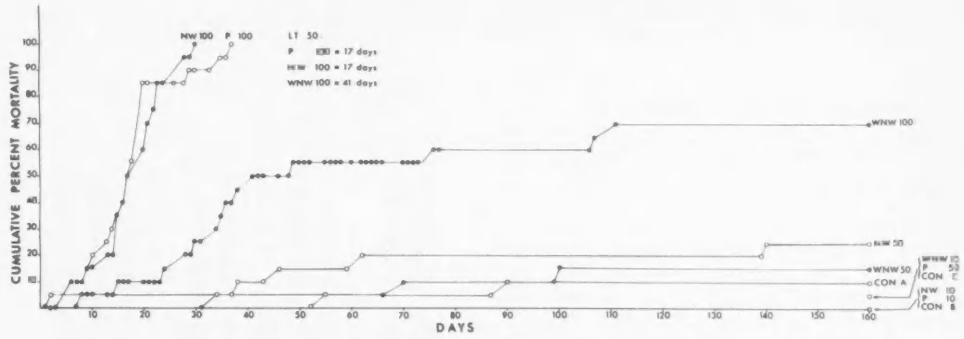


FIG. 1. Cumulative mortality of juvenile *M. entomon* during chronic exposure to different concentrations of water-soluble fractions of Pembina (P), Norman Wells (NW), and weathered Norman Wells (WNW) crudes. Number following oil abbreviation indicates concentration as percent of stock solution. Three control groups are indicated as CON A, B, C. Data points are omitted when cumulative mortality remains unchanged. The times for 50% of the animals to die (LT50) are indicated on the figure.

of the difference in duration of control and oil-exposed groups, the duration of each intermolt period of each exposure group is statistically compared with that of the corresponding intermolt period of the combined control groups.

Animals exposed to the 100% extract of weathered NW crude (1.01 ppm) exhibited a consistent and pronounced change in the duration of the intermolt periods. The first period measured was almost twice as long as that of the controls (51.3 ± 12.1 d and 25.9 ± 4.0 d, respectively). The length of the period progressively decreased with each succeeding molt until by the third intermolt period it was only 17% longer than that of the controls. Lower concentrations of weathered NW crude had no significant effect. None of the WSF's from the other oils caused a consistent change in lengths of all of the intermolt periods. However, in animals exposed to extracts of fresh NW (0.19–0.90 ppm) and P (0.26 ppm) crudes early intermolt periods were either comparable in length to, or slightly longer than, those of controls, while later periods were significantly shorter than controls.

### EFFECTS ON GROWTH

After an 84-d exposure to the oil only three test groups had mean lengths significantly less than that of the controls (Fig. 2A). Animals exposed to the 100% extract of weathered NW crude (1.01 ppm) were most severely affected; the mean length was only 60% that of the controls. Mean lengths of animals exposed to the 10% extract of weathered NW (0.10 ppm) and the 50% extract of fresh NW (0.90 ppm) crudes were about 12% less than that of the controls. After 160 d of exposure the mean lengths of four of the test groups were significantly different from the controls. As before, animals exposed to the 50% NW (0.90 ppm) and 100% weathered NW (1.01 ppm) extracts were significantly shorter. However, in the case of the animals exposed to weathered NW the difference in length from the controls was considerably less after 160 d (8.9%) than it was after 84 d (39.1%).

Lengths of animals exposed to 50% weathered NW (0.52 ppm) and 10% P (0.04 ppm) extracts for 160 d were slightly greater than that of the controls. In the latter case a slight stimulation of growth was also observed after an 84-d exposure.

In only two of the exposure groups was the mean dry weight of the animals, after 160 d of exposure, significantly different from that of the controls (Fig. 2B). The weight of animals exposed to the 100% WSF of weathered NW (1.01 ppm) was less than half that of the controls, while the weight of those exposed to the 50% P extract (0.26 ppm) was about 14% less than that of the controls.

## Discussion

There is no evidence in the present study that *M. entomon* is especially sensitive to petroleum during ecdysis. None of the animals exposed to the 100% WSF's of the three oils died during this process. In contrast, several studies have demonstrated that some crustaceans are more sensitive to petroleum during ecdysis than during the intermolt period (Mecklenberg et al. 1977; Wells and Sprague 1976). Karinen and Rice (1974) found that if adult tanner crabs were exposed to oil just prior to a molt they often died during ecdysis. Lockwood (1967) and others have suggested that increased sensitivity to stress during ecdysis may be a general phenomenon among crustaceans. Mecklenberg et al. (1977) speculated that the increased permeability of the exoskeleton at this time could facilitate penetration of toxic compounds into the tissues and that reduction in metabolism might decrease the ability of the animal to metabolize and excrete toxic compounds.

TABLE 2. Intermolt duration (days) of *M. entomon* juveniles chronically exposed to different concentrations of dissolved hydrocarbons extracted from crude oils. (ΔC, difference in days from combined controls; %ΔC, percent difference from combined controls.)

| Oil | % of stock | Intermolt no. | *N* | $\bar{x}$ | SD | SE | ΔC | %ΔC | *t* | *P* |
|---|---|---|---|---|---|---|---|---|---|---|
| NW | 100 | 1 | 0 | — | — | — | — | — | — | — |
| | 50 | 1 | 17 | 30.4 | 7.5 | 1.8 | 4.5 | +17.4 | −3.20 | 0.005 |
| | | 2 | 16 | 24.8 | 2.2 | 0.6 | 1.2 | +5.1 | −1.77 | 0.05 |
| | | 3 | 16 | 25.7 | 3.2 | 0.8 | −1.6 | −5.9 | 1.36 | 0.10 |
| | | 4 | 13 | 27.6 | 3.6 | 1.0 | −6.3 | −22.8 | 3.01 | 0.005 |
| | | 5 | 4 | 38.0 | 4.1 | 2.0 | −6.6 | −14.8 | 1.42 | 0.10 |
| | 10 | 1 | 20 | 25.6 | 3.5 | 0.8 | −0.3 | −1.2 | 0.29 | NS |
| | | 2 | 20 | 24.8 | 4.4 | 1.0 | 1.2 | 5.1 | −1.50 | 0.10 |
| | | 3 | 20 | 24.5 | 2.5 | 0.6 | −2.8 | −10.3 | 2.71 | 0.005 |
| | | 4 | 19 | 29.3 | 3.3 | 0.7 | −4.6 | −13.6 | 2.64 | 0.01 |
| | | 5 | 8 | 44.1 | 9.2 | 3.2 | −0.5 | −1.1 | 0.11 | NS |
| WNW | 100 | 1 | 7 | 51.3 | 12.1 | 4.6 | 25.4 | 98.1 | −11.35 | 0.005 |
| | | 2 | 7 | 34.3 | 7.4 | 2.8 | 10.7 | 45.3 | −7.89 | 0.005 |
| | | 3 | 5 | 32.0 | 8.0 | 3.6 | 4.7 | 17.2 | −2.10 | 0.025 |
| | | 4 | 1 | 27.0 | — | — | — | — | — | — |
| | 50 | 1 | 18 | 27.6 | 3.8 | 0.9 | 1.7 | 6.6 | −1.57 | 0.10 |
| | | 2 | 18 | 23.7 | 2.5 | 0.6 | 0.1 | 0.4 | −0.15 | NS |
| | | 3 | 18 | 26.1 | 2.8 | 0.7 | −1.2 | −4.4 | 1.10 | NS |
| | | 4 | 17 | 33.2 | 4.4 | 1.1 | −0.7 | −2.1 | 0.37 | NS |
| | | 5 | 6 | 41.0 | 4.6 | 1.9 | −3.6 | −8.1 | 0.91 | NS |
| | 10 | 1 | 20 | 27.7 | 6.9 | 1.5 | 1.8 | 6.9 | −1.39 | 0.10 |
| | | 2 | 20 | 24.9 | 5.0 | 1.1 | 1.3 | 5.5 | −1.51 | 0.10 |
| | | 3 | 19 | 28.5 | 5.4 | 1.2 | 1.2 | 4.4 | −0.97 | NS |
| | | 4 | 15 | 37.9 | 13.5 | 3.7 | 4.0 | 11.8 | −1.49 | 0.10 |
| | | 5 | 1 | 56.0 | — | — | — | — | — | — |
| P | 100 | 1 | 0 | — | — | — | — | — | — | — |
| | 50 | 1 | 19 | 26.7 | 3.0 | 0.7 | 0.8 | 3.1 | −0.79 | NS |
| | | 2 | 19 | 26.1 | 5.3 | 1.2 | 2.5 | 10.6 | −2.77 | 0.005 |
| | | 3 | 19 | 25.7 | 3.4 | 0.8 | −1.6 | −5.9 | 1.45 | 0.10 |
| | | 4 | 19 | 30.5 | 4.6 | 1.1 | −3.4 | −10.0 | 1.89 | 0.05 |
| | | 5 | 6 | 47.2 | 13.0 | 5.3 | 2.6 | 5.8 | −0.43 | NS |
| | 10 | 1 | 20 | 22.7 | 2.3 | 0.5 | −3.2 | −12.4 | 3.34 | 0.005 |
| | | 2 | 20 | 22.5 | 3.4 | 0.8 | −1.1 | −4.7 | 1.55 | 0.10 |
| | | 3 | 20 | 26.5 | 3.6 | 0.8 | −0.8 | −2.9 | 0.73 | NS |
| | | 4 | 20 | 32.7 | 5.0 | 1.1 | −1.2 | −3.5 | 0.66 | NS |
| | | 5 | 6 | 43.3 | 6.2 | 2.5 | −1.3 | −2.9 | 0.31 | NS |
| Controls | | | | | | | | | | |
| CON A | 0 | 1 | 19 | 25.7 | 4.5 | 1.0 | | | | |
| | | 2 | 18 | 24.8 | 2.9 | 0.7 | | | | |
| | | 3 | 17 | 28.9 | 4.4 | 1.1 | | | | |
| | | 4 | 15 | 35.1 | 7.8 | 2.0 | | | | |
| | | 5 | 3 | 47.7 | 1.5 | 0.9 | | | | |
| CON B | 0 | 1 | 20 | 25.6 | 3.6 | 0.8 | | | | |
| | | 2 | 20 | 23.1 | 2.2 | 0.5 | | | | |
| | | 3 | 20 | 25.7 | 4.1 | 0.9 | | | | |
| | | 4 | 18 | 31.1 | 4.1 | 1.0 | | | | |
| | | 5 | 2 | 41.0 | 18.4 | 13.0 | | | | |
| CON C | 0 | 1 | 18 | 26.4 | 4.1 | 1.0 | | | | |
| | | 2 | 19 | 22.8 | 1.7 | 0.4 | | | | |
| | | 3 | 19 | 27.6 | 4.0 | 0.9 | | | | |
| | | 4 | 18 | 35.6 | 8.5 | 2.0 | | | | |
| | | 5 | 3 | 44.0 | 5.0 | 2.9 | | | | |
| Combined controls | 0 | 1 | 57 | 25.9 | 4.0 | 0.5 | | | | |
| | | 2 | 57 | 23.6 | 2.4 | 0.3 | | | | |
| | | 3 | 56 | 27.3 | 4.3 | 0.6 | | | | |
| | | 4 | 51 | 33.9 | 7.2 | 1.0 | | | | |
| | | 5 | 8 | 44.6 | 8.0 | 2.8 | | | | |

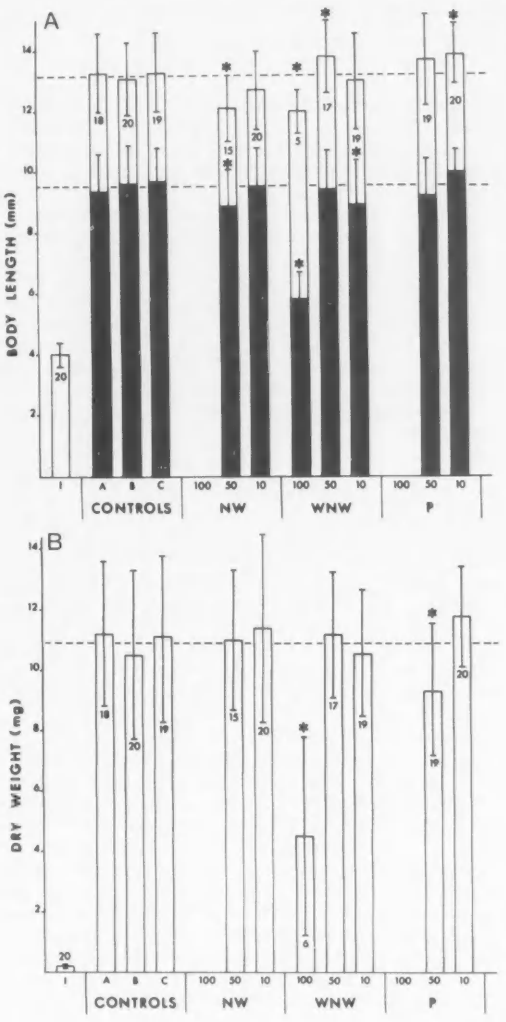


FIG. 2. Changes in body length (A) and dry weight (B) of juvenile *M. entomon* after chronic exposure to WSF's of crude oils. Histogram shows mean length and weight after 84-d (dark portion of bar) and 160-d (light portion of bar) exposure. Vertical lines indicate standard deviations. Number of animals measured at the termination of the experiment indicated within each bar. Bar marked I indicates mean length or weight at the commencement of the exposure period. Broken lines indicate mean lengths or weights of combined controls after 84 d (*lower*) and 160 d (*upper*). Asterisk indicates difference significant at $P > 0.05$ level.

Exposure of *M. entomon* to even the highest concentrations of the oils did not cause a delay in the onset of the next ecdysis. In fact, exposure to the 50 and 100% extracts of weathered Norman Wells and Pembina crudes resulted in a slight acceleration, while exposure to lower concentrations, or to any concentration of fresh Norman Wells crude, had little effect. In contrast, exposure of king crab larvae to Cook Inlet crude at concentrations comparable to that in the 100% WSF's used in the present study resulted in a complete inhibition of molting (Mecklenberg et al. 1977). However, exposure of the crabs to lower concentrations for shorter periods resulted in an acceleration of the onset of ecdysis.

The effect of the oils on the duration of intermolt periods was rather variable and in only a few cases were there consistent trends. The most dramatic prolongation of the intermolt period occurred in animals exposed to the 100% WSF's of Norman Wells crude. The first intermolt period measured was nearly twice as long as normal. The duration of the next two periods was reduced by approximately half each time. This decline in the length of the intermolt period is not attributable to the death of the less robust members of the population because the phenomenon was observed for individual animals as well as in the mean values for the population. This may represent a physiological adaptation to the oil by the surviving members of the population. Animals exposed to the 50% WSF of fresh Norman Wells crude also exhibited a marked prolongation of the first two intermolt periods, while subsequent periods were significantly shorter than normal.

Growth and the molt cycle are closely associated processes in crustaceans and stresses influencing either can be expected to cause a corresponding change in the other. The most pronounced change in growth occurred in animals exposed to the 100% WSF of weathered Norman Wells crude. In the first 84 d of exposure the length increased by 1.79 mm, compared with an increment of 5.52 mm for the control animals. Between 84 and 160 d the growth in the exposed group amounted to 6.20 mm, compared with 3.64 mm in the control group. Not only does the growth of the oil-exposed animals increase during the latter part of the exposure period, but it exceeds that of the control group. A similar recovery trend occurred in the duration of successive intermolt periods in this exposure group. The changes in growth in all of the other groups were relatively minor.

In the majority of studies with crustaceans little change in growth could be demonstrated as a consequence of exposure to petroleum hydrocarbons (Anderson 1976; Anderson et al. 1974; Caldwell et al. 1977; and others). In a few species a significant reduction in growth after chronic exposure to oil has been reported (Tatem 1977; Linden 1976; Anderson 1976).

This study suggests that growth and molting are not particularly good indicators of oil stress in *Mesidotea*. Major changes in growth rate and in the molt cycle only occur at oil concentrations approximating the chronic lethal level. At lower concentrations changes in growth are slight and probably of little ecological significance. Since the animals spend much of their time partially buried in the sediments, and also appear

to ingest considerable quantities of sediment, it would be worthwhile to examine sublethal effects of chronic exposure to oil contaminated sediments.

## Acknowledgments

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# Bioavailability of Crude Oil from Experimentally Oiled Sediments to English Sole (*Parophrys vetulus*), and Pathological Consequences[1]

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McCain, B. B., H. O. Hodgins, W. D. Gronlund, J. W. Hawkes, D. W. Brown, M. S. Myers, and J. H. Vandermeulen. 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole (*Parophrys vetulus*), and pathological consequences. J. Fish. Res. Board Can. 35: 657–664.

English sole (*Parophrys vetulus*) were exposed to experimentally oiled (Alaskan North Slope crude oil) sediments over a 4-mo period to assess the bioavailability and tissue hydrocarbon distribution kinetics in flatfish. Data were also obtained on hydrocarbon related physiological changes and tissue pathology. Crude oil was mixed with aromatic hydrocarbon-free sediments to a concentration of 700 μg/g dry weight at the beginning of the experiment. During the 1st mo of the experiment this concentration decreased to 400 μg/g dry weight, and remained relatively stable during the remainder of the 4-mo period. Compositional changes were observed in the alkane and aromatic fractions, with a differential decrease in the substituted naphthalenes. Flatfish maintained in such oiled sediments readily took up alkane and aromatic petroleum hydrocarbons from these sediments, and accumulated these in skin, muscle, and liver; 1- and 2-methylnaphthalene and 1,2,3,4-tetramethylbenzene were accumulated to greater extent than other aromatics. Tissue hydrocarbons decreased with time, and after 27-d continuous exposure to oiled sediments only the liver contained detectable levels of hydrocarbons. After 2 mo $<2\%$ of the initial aromatic hydrocarbon load could be detected, and only in the liver in flatfish that were continuously maintained on oiled sediments. Depuration of tissue aromatics differed for various aromatics, 1,2,3,4-tetramethylbenzene and 2-methylnaphthalene being most persistent. Depuration is thought to be due to induction of the aryl hydrocarbon hydroxylase system during initial exposure to oiled sediments. Concomitant with the high tissue hydrocarbon period were found enhanced weight loss and severe hepatocellular lipid vacuolization (HLV). Although the observations on growth changes and liver pathology are preliminary, the data indicate the need for further detailed study of fish growth abnormalities and pathology in the presence of petroleum hydrocarbons.

*Key words:* flatfish, crude oil, bioavailability, pathology, aromatic hydrocarbons, bottom sediments, depuration

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Nous avons exposé des limandes soles (*Parophrys vetulus*) à des sédiments expérimentalement traités au pétrole (pétrole brut de versant nord de l'Alaska) sur une période de 4 mo, dans le but d'évaluer la biodisponibilité et la cinétique de distribution des hydrocarbures dans le tissu des poissons plats. Nous avons également recueilli des données sur les changements physiologiques et la pathologie tissulaire associés aux hydrocarbures. Au début de l'expérience, nous avons mélangé du pétrole brut avec des sédiments libres d'hydrocarbures à une concentration de 700 μg/g de poids sec. Pendant le premier mois

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de l'expérience, cette concentration a passé à 400 μg/g de poids sec et est demeurée relativement stable durant tout le reste de la période de 4 mo. Nous avons observé des changements de composition des fractions alkanes et aromatiques, ainsi qu'une diminution différentielle des naphtalènes substitués. Les poissons plats maintenus dans des sédiments ainsi affectés par le pétrole absorbent facilement les hydrocarbures de pétrole alkanes et aromatiques à partir de ces sédiments et les accumulent dans la peau, le muscle et le foie; ils accumulent 1- et 2-méthylnaphtalène et 1,2,3,4-tétraméthylbenzène plus que tous les autres aromatiques. Les hydrocarbures du tissu diminuent avec le temps et, après 27 jours d'exposition continue aux sédiments affectés par le pétrole, seul le foie contient des niveaux décelables d'hydrocarbures. On peut déceler, après 2 mo, 2% de la charge initiale d'hydrocarbures aromatiques et cela, seulement dans le foie des poissons plats maintenus continuellement dans les sédiments affectés par le pétrole. L'épuration des aromatiques du tissu se fait différemment selon les aromatiques, le 1,2,3,4-tétraméthylbenzène et le 2-méthylnaphtalène étant les plus persistants. On croit que l'épuration est causée par induction du système aryl hydrocarbures hydroxylase lors de l'exposition initiale aux sédiments contenant du pétrole. Une forte période d'hydrocarbures dans le tissu est accompagnée d'une perte de poids plus prononcée et d'une forte vacuolisation des lipides hépatocellulaires (HLV). Bien que ces observations sur les changements dans la croissance et la pathologie du foie soient préliminaires, ils font quand même ressortir le besoin d'études plus poussées des anomalies de la croissance et de la pathologie des poissons en présence d'hydrocarbures de pétrole.

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BEACH and bottom sediments are now generally recognized as the final repository of certain components of the oil entering the water column as a result of oil spills and oil pollution (e.g. Blumer and Sass 1972; Kolpack et al. 1971; Vandermeulen and Gordon 1976). With concentrations of petroleum derived hydrocarbons up to 3805 μg/g reported for postspill sediments (Gilfillan and Vandermeulen 1978) concern has been focused on the bioavailability of these long-term residual hydrocarbons to organisms residing in or on the bottom sediments. This is particularly relevant since there is experimental evidence that certain hydrocarbons and trace metals contained in petroleum products inhibit disease-resistance mechanisms in mammals (Stjernswärd 1974; Kripke and Weiss 1970) and in fish (Robohm and Nitkowski 1974). Considerable evidence also exists that certain hydrocarbons and associated trace metals are carcinogens (Hueper and Payne 1960; Bingham et al. 1965; Wallcave et al. 1971; Sullivan 1974).

This paper describes the compositional changes of Alaskan crude oil mixed in with uncontaminated sediment, and is the first account of the bioavailability of petroleum hydrocarbons from oiled sediments to the English sole (*Parophrys vetulus*). A species of flatfish was selected for these studies because (1) these fish spend nearly their entire life burrowed into bottom sediments (Clemens and Wilby 1961) and (2) flatfish are known to accumulate petroleum hydrocarbons in their tissues (Roubal et al. 1978).

This study further presents preliminary data on growth deterioration and liver pathology in sole maintained on experimentally oiled sediments for 4 mo.

## Materials and Methods

### EXPERIMENTAL SETUP

Sediments for this study were collected from a beach near Sequim, Wash. They were frozen and thawed 3 times so as to kill as many interstitial organisms as possible, and were then kept frozen until needed.

A 0.2% (vol/vol) mixture of Alaskan crude oil (Alaskan North Slope, Sadlerochit) and sediment was prepared by mixing a sediment–oil–seawater slurry in a commercial cement mixer lined with fiberglass. The oiled sediment was then placed in a specially constructed aquarium (Fig. 1) to a depth of 5 cm. A similar aquarium containing unoiled sediment served as a control. The flatfish were added to both aquaria after the sediments were rinsed overnight in running seawater.

English sole (*P. vetulus*) were taken with a beach seine 32 km south of Seattle, Wash., in Puget Sound. Previous investigations had shown fish from this site to have very low levels of pathological abnormalities (Wingert 1977). The fish were cold-branded for identification purposes, and were divided into an experimental and a control group of 35 fish each. They were kept in laboratory holding tanks for 8 d before use.

Control and experimental groups were similar in all respects, with male–female ratios of 1.5 and 1.2, respectively. They consisted of two size-classes in both groups — small and large. Mean lengths in the control group were 146.1 ± 11.5 and 242.3 ± 20.3 mm, respectively, with mean weights 25.62 ± 6.36 and 118.48 ± 31.23 g, respectively. In the experimental group mean lengths were 145.5 ± 11.7 and 244.2 ± 23.0 mm, respectively, and mean weights 24.60 ± 5.87 and 119.41 ± 34.16 g, respectively. The fish were maintained in flowing seawater (flow rate 10–12 L/min, average water temperature 8.5°C, salinity 27‰) with an 11-h-light and 13-h-dark cycle. Fish were fed earthworms once daily to satiation. Uneaten food was removed after 12 h.

### HYDROCARBON ANALYSIS

Hydrocarbon analysis was done at the NOAA National Analytical Facility (NAF), Seattle, Wash., with a modification of a procedure described by MacLeod et al. (1976). Sediment analysis included gravimetric determination of total extractable hydrocarbons and gas–liquid chromatography (GLC) for alkanes and arenes. Total extractable

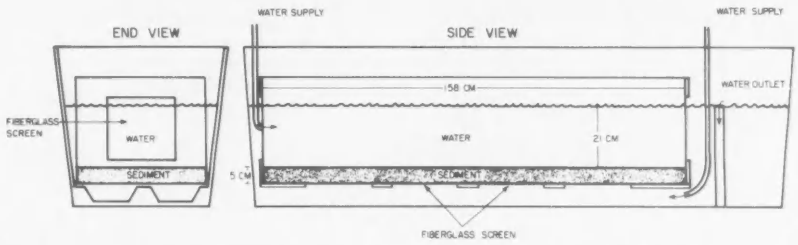


FIG. 1. Aquaria used in experiments with flatfish maintained continuously for 4 mo on crude oil contaminated sediments.

petroleum-derived hydrocarbons (TEPH) were determined by subtracting the concentration of total extractable hydrocarbons in the control sediment from that in the oiled sediment. Untreated control sediment contained no aromatic hydrocarbons, and between 1 and 36 $\mu$g/g concentrations of saturated hydrocarbons. In addition, the sediment contained iron, copper, and zinc in concentrations of 1210.0, 11.5, and 9.0 mg/kg dry weight, respectively. Eighty-seven percent of the sediment particles was between 0.18 and 0.84 mm in diameter.

To monitor hydrocarbon changes in the sediment-bound crude oil, 400-g samples of sediment were taken immediately after mixing and at various intervals subsequently throughout the 4-mo experiment. Samples were taken from the top 2 cm of the sediment with a methylene-chloride-rinsed beaker, after partial draining of the aquarium.

To monitor uptake of crude oil hydrocarbons into the tissues of the flatfish, three fish were killed from both the control and experimental tanks at the beginning of the experiment and at six subsequent periods during the 4-mo experiment. Samples of skin, muscle and liver were analyzed for petroleum hydrocarbons by GLC (MacLeod et al. 1976). The muscle samples were analyzed individually, but the skin and liver samples were pooled for petroleum hydrocarbon assay. Tissue samples were kept frozen at −20°C until analysis.

### PATHOLOGY

At the same time that fish tissues were taken for hydrocarbon analysis, tissue samples were collected for hematological, histological, and ultrastructural study. Hematology included determination of hemoglobin concentrations (cyanomethemoglobin method, Oser 1965), hematocrit, total red blood cell and leucocyte counts, and differential leucocyte counts.

For pathology, specimens of liver tissue were prepared for electron microscopy by the method of Hawkes (1974). Thin sections were triple stained with lead citrate, uranyl acetate, and again in lead citrate.

## Results

### SEDIMENT HYDROCARBONS

Although initially the oil–sediment mixture was prepared at a 0.2% (vol/vol) concentration, this dropped immediately to 700 $\mu$g/g after the overnight rinse with running seawater before addition of the test fish. During the entire 4-mo exposure period the total sediment oil concentration continued to decrease, at first rapidly for the 1st mo, and then more slowly thereafter (Fig. 2). During the first 30 d of the experiment total sediment hydrocarbon concentrations (TEPH) decreased from 700 to 400 $\mu$g/g dry weight. Subsequently for the remainder of the experiment the concentration remained relatively stable, decreasing slightly to ca. 350 $\mu$g/g dry weight. During the course of the experiment a steep concentration gradient developed between the top 1 cm and the lower 4 cm of the sediment layer. Thus at the end of the experiment crude oil concentration in the top surface sediments was ca. 43 $\mu$g/g (dry weight), while concentration in a sample taken from the bottom of the sediment layer averaged ca. 540 $\mu$g/g dry weight.

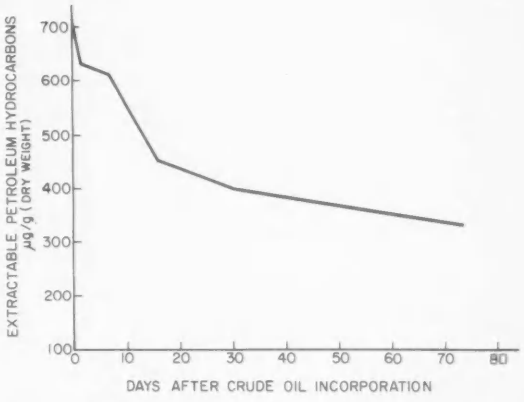


FIG. 2. Time course of total extractable crude oil derived hydrocarbons in the surface layer of experimentally oiled sediments.

Analysis for certain specific aromatic hydrocarbons showed initial high concentrations of the methyl-substituted naphthalenes relative to unsubstituted naphthalene (Table 1). During the first 30 d of the experiment total aromatic content of the sediment decreased by ~30%. The various aromatic components did not decrease equally, however, with marked persistence of the 1- and 2-methylnaphthalenes and of fluorene.

TABLE 1. Aromatic petroleum hydrocarbon concentrations in experimentally oiled sediments and changes over time over a 30-d period. Samples were taken from the top 2 cm of a 5-cm sediment layer.

| | Hydrocarbon concn (ng/g dry wt) | | | | | | |
|---|---|---|---|---|---|---|---|
| Hydrocarbons | day 0 | % composition at | day 2 | day 7 | day 16 | day 30 | % remaining on day 30 |
| 1,2,3,4-tetramethylbenzene | 105 | 4 | 74 | 50 | 64 | 61 | 58 |
| Naphthalene | 210 | 8 | 200 | 61 | 89 | 82 | 39 |
| 1-methylnaphthalene | 408 | 16 | 736 | 400 | 362 | 557 | 137 |
| 2-methylnaphthalene | 753 | 29 | 742 | 601 | 723 | 519 | 69 |
| 2,6-dimethylnaphthalene | 375 | 14 | 185 | 411 | 344 | 218 | 58 |
| 2,3,5-trimethylnaphthalene | 379 | 15 | 338 | 318 | 336 | 187 | 49 |
| Fluorene | 87 | 3 | 75 | 67 | 78 | 102 | 117 |
| Phenanthrene | 275 | 11 | 155 | 150 | 156 | 112 | 41 |

TABLE 2. Petroleum hydrocarbons in the tissues of English sole exposed to oil-contaminated sediment (test, T) and to nonoiled sediment (control, C) for 2 mo continuously.

| | Concn (ng/gm dry wt)[b] | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 11 d | | | | | | 27 d | | | | | | 51 d | | | | | |
| | Skin | | Muscle | | Liver | | Skin | | muscle | | Liver | | Skin | | Muscle | | Liver | |
| Polycyclic aromatic hydrocarbons[a] | C | T | C | T | C | T | C | T | C | T | C | T | C | T | C | T | C | T |
| 1,2,3,4-tetramethylbenzene | — | 33 | — | 44 | — | 922 | — | — | — | — | — | 863 | — | — | — | — | — | 124 |
| Biphenyl | — | 156 | — | — | — | 307 | — | — | — | — | — | 278 | — | — | — | — | — | — |
| Naphthalene | — | 82 | — | 20 | — | 100 | — | — | — | — | — | — | — | — | — | — | — | — |
| 1-methylnaphthalene | — | 1189 | — | 369 | — | 1940 | — | — | — | — | — | 1325 | — | — | — | — | — | — |
| 2-methylnaphthalene | — | 888 | — | 279 | — | 3070 | — | — | — | — | — | 1500 | — | — | — | — | — | 60 |
| 2,6-dimethylnaphthalene | — | 130 | — | — | — | 69 | — | — | — | — | — | — | — | — | — | — | — | — |

[a]Arenes with aromatic rings ranging from one to six, i.e. *o*-xylene to benz[*a*]anthracene, were determined and only the compounds listed here were found in significantly higher concentrations in test fish.

[b]Skin and liver samples were pooled from three fish at each analysis; muscle samples were analyzed individually.

## TISSUE HYDROCARBONS

Alkanes and aromatics were detectable in the tissues of fish exposed to the oiled sediments. Aromatics were found in the skin, muscle, and liver of all oil-exposed fish, but the concentrations and the distribution declined with time, despite the presence of crude oil in the surrounding oiled sediments. After 11-d exposure the liver, skin, and muscle of oil-exposed fish contained significant concentrations of a range of aromatics (Table 2), of these 1,2,3,4-tetramethylbenzene, and the 1- and 2-methylnaphthalenes were the most abundant. After 27-d exposure aromatic hydrocarbons were found only in the liver, most notably the substituted benzene and the 1- and 2-methylnaphthalenes. After 51-d exposure the tissue aromatic hydrocarbons concentrations had dropped to $<2\%$ of the 11-d values, and consisted only of the tetramethylbenzene and the 2-methylnaphthalene. Of these the tetramethylbenzene was the most persistent with 13% of the original tissue load detectable in the liver after 51-d exposure.

The concentrations of aromatics found in the various tissues throughout this experiment were of the same order of magnitude as hydrocarbon concentrations in the oiled sediments.

Alkane analysis of the fish tissues revealed a significant uptake of alkanes as well. After 11 d concentrations of several alkanes ($C_{11}$, $C_{13}$, $C_{17}$, $C_{26}$, $C_{27}$, $C_{28}$, and $C_{31}$) as much as fivefold higher than tissues from control fish were observed. After 27 and 51 d of exposure, however, the alkane levels in oil-exposed fish were not significantly different from those in control fish.

## PATHOLOGY

Slight but consistent behavioral differences were observed between the two groups of flatfish. The oil-exposed group appeared less active than the control group. Also, the oil-exposed fish did not feed as well as the controls, with larger amounts of uneaten food removed daily following feeding.

Both groups of fish experienced weight loss on being brought into the laboratory, up to 4% in the control

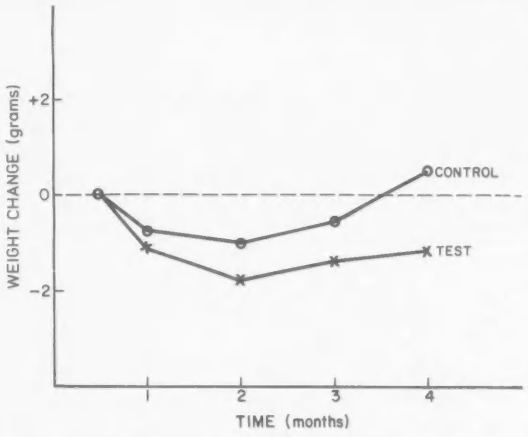


FIG. 3. Weight changes in oil-exposed (test) and control English sole over 4 mo. Each observation at monthly intervals represents the average change (grams) from weights at 2 wk for 16 test and 15 control fish.

group versus a maximum loss of 8% in the experimental group. Weight lost was regained, however, by the control fish during the 3rd mo (Fig. 3). In contrast, the oil-exposed group differed from the control group, in greater weight loss and in slower prolonged weight recovery subsequently. The numbers of fish gaining and not gaining weight after 4 mo differed significantly between the experimental and control groups ($P = 0.05$). There were no mortalities during the 4-mo experiment. However, by the end of the experiment three of the oil-exposed fish were emaciated, but with no evidence of microbial infection. Subsequently one of these died and the other two were moribund.

Hematocrit and hemoglobin levels also differed significantly ($P < 0.01$) between oil-exposed and control fish during the 1st mo of the experiment. Hematocrit and hemoglobin levels in oil-exposed fish ($20.06 \pm 1.7\%$ and $5.5 \pm 0.7$ mg/dL, respectively) were higher than in control fish ($16.08 \pm 0.9\%$ and $4.2 \pm 0.2$ mg/dL). After 51 d the elevated levels in the oil-exposed fish had decreased and were no longer significantly different from controls. There were no measurable differences between the two groups with respect to red blood cell and leucocyte counts and differential leucocyte counts.

No histological differences were seen in spleen, kidney, intestine, fins, gills, and skin of fish from both groups. Fish from both groups had various liver abnormalities. However, certain severe liver abnormalities were found only in the oil-exposed fish, especially during the first 27 d of the experiment, that were not seen in control fish. During the 1st mo livers from oil-exposed fish showed severe hepatocellular lipid vacuolization (HLV), with >95% of the liver cytoplasm taken up by lipid droplets and lipid vacuoles (Fig. 4 and 5). Extensive proliferation of whorls of rough endoplasmic reticulum was typical of all such hepatocytes and livers and filled the cell spaces otherwise not occupied by lipid vacuoles (Fig. 5).

## Discussion

Clearly, sediment-bound petroleum hydrocarbons are taken up by flatfish under these experimental conditions, and become distributed throughout their tissues. Of interest is the process of bioavailability in that it appears to be dependent on a number of factors including aromatic structure, bioaccumulation, and degradation mechanisms in the flatfish tissues.

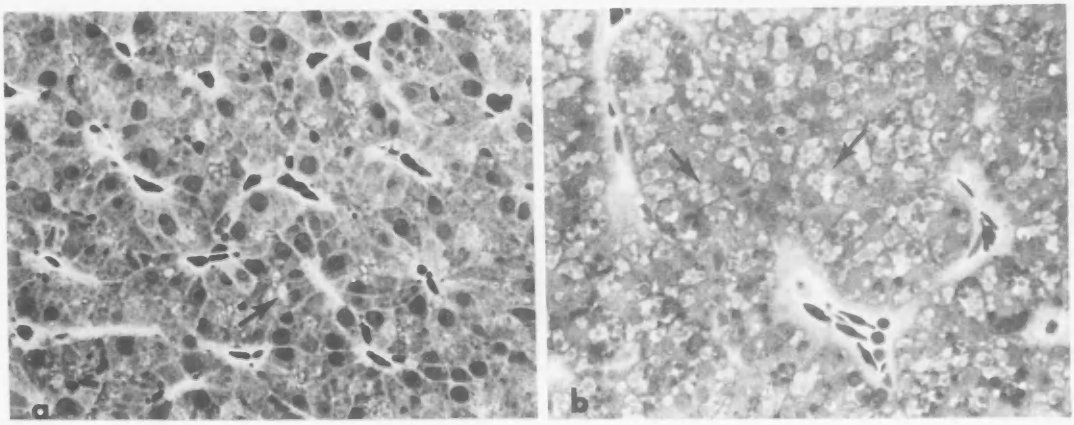


FIG. 4. a, Light micrograph of plastic-embedded liver tissue from a control English sole. Lipid vacuolization (arrow) is moderate. Richardson's stain. × 648
b, Light micrograph of hepatocytes from an English sole exposed for 27 d to crude oil contaminated sediments. The lipid vacuolization (arrows) is of the most severe type. Richardson's stain. × 648

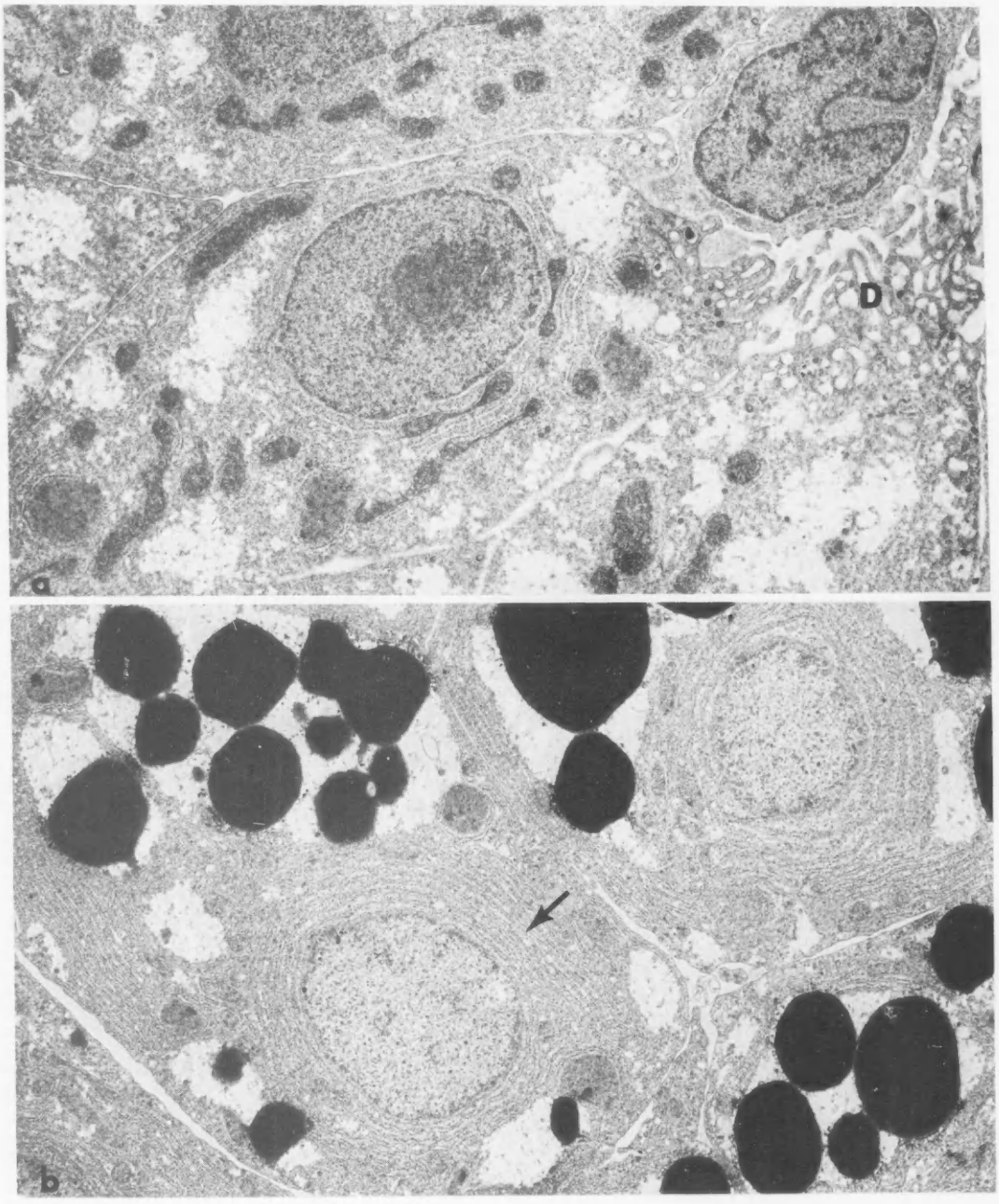


FIG. 5. a, Electron micrograph of liver cells from control English sole. These hepatocytes border a sinusoid, and one face of the cells forms the space of Disse (D). × 3441
b, Electron micrograph of hepatocytes from English sole exposed to oiled-sediments for 27 d. Lipid droplets (L) and layers of extensive endoplasmic reticulum (arrow) are prominent features. × 2511

That bioavailability is a function of aromatic structure is suggested by the differential uptake and retention of the various aromatics assayed, with the 1- and 2-methylnaphthalenes and the tetramethylbenzene taken up to a greater extent than any of the other aromatics. Such differential uptake is not unique, having been described for other systems (e.g. brown shrimp, Anderson et al. 1974; coho salmon and starry flounder, Roubal et al. 1978). This does suggest, however, that this phenomenon is a common feature of the uptake mechanism of petroleum hydrocarbons by tissues. It is noteworthy that of these favored substituted aromatics the 2-methylnaphthalene and the 1,2,3,4-tetramethylbenzene are the most persistent aromatics, being detected in liver tissues up to 51 d into the experiment.

The second aspect of bioavailability, i.e. bioaccumulation, is indicated by the high levels of crude oil aromatics detected in the tissues throughout the first 51 d of the experiment. Tissue hydrocarbon levels are of the same order of magnitude as those found in the sediment, and suggest a very active uptake mechanism. Again, this is not a unique observation but confirms the work of Roubal et al. (1978) who observed bioaccumulation of $C_4$- and $C_5$-substituted benzenes from 0.9 ppm Prudhoe Bay crude oil by starry flounder, and calculated a bioaccumulation factor of greater than 11 000 for these compounds from the water column. The present data extend the observation of the bioaccumulation phenomenon to uptake from oiled sediments, and indicate that this also is a persistent feature of the bioavailability process.

The third feature of bioavailability, that of potential simultaneous biodegradation, is suggested by the disappearance of tissue-bound crude oil hydrocarbons after an initial high tissue load, despite the fact that the surrounding oiled sediments continue to remain oiled, at ca. 350 $\mu$g/g dry weight. Two mechanisms acting simultaneously can be evoked to account for this clearing of hydrocarbons from the oil-exposed fish tissues. Presumably the hydrocarbons found in the skin and muscle are removed via the liver. The process in the liver is less well understood, but possibly involves induction of the mixed function oxygenases (aryl hydrocarbon hydroxylase system) shown to be involved in hydrocarbon degradation in teleost tissues (Payne and Penrose 1975a, b). If this is in fact the mechanism underlying this disappearance of the tissue aromatics, then it is interesting to note both the time scale of degradation, and the selective pattern of degradation with a graded degradation first of naphthalene and its dimethyl-substituted derivative, then in turn of biphenyl and the 1-methyl-substituted naphthalene, and finally the 2-methyl-substituted naphthalene and the 1,2,3,4-tetramethylbenzene compound. The implication of course is that although the biodegradative potential of these tissues is effective and efficient in removing these foreign compounds from tissues, and can respond relatively quickly to influx of these compounds, nonetheless the persistence of two of these aromatic compounds suggests a potential long-term concern.

Turning to the observed physiological and pathological abnormalities the situation is less clear-cut. The experimental conditions were not ideal, with both control and oiled fish experiencing weight loss during the first part of the 4-mo exposure experiment. This is not unusual and can be expected in flatfish brought from the wild into the laboratory (Fletcher personal communication). The fact remains, however, that despite the observed weight loss the continuously oiled group experienced a significantly greater weight loss, showed significantly higher hematocrit and hemoglobin levels, and experienced more severe hepatocellular lipid vacuolization (HLV) in the liver tissues. Taken together these observations do suggest that the oiled flatfish experienced a greater degree of stress than did the control group. It is important, we think, to point out here that these abnormalities or differences were greatest during the 1st mo of the experiment, when both sediment and tissue hydrocarbon levels also were at their highest. Subsequently hydrocarbon levels declined. Thus for example both the frequency of severe HLV and the tissue aromatic hydrocarbon load declined markedly during the 2nd and 3rd mo of the experiment. Such correlation between HLV and exposure of marine fish to pollutants has also been reported in English sole from a polluted urban estuary (McCain et al. 1977); in *Microstomus pacificus* and *Leptocephalus zacharius* from Coal Oil Point, an area in southern California with a natural oil seep (K. V. Pierce personal communication); and in spot (*Lepostomus xanthurus*) exposed to polychlorinated biphenyls (Couch 1975). The HLV observations presented here further suggest that severe HLV may be a nonspecific liver lesion induced by a variety of pesticides and PCBs in a variety of organisms (viz. also Couch 1975).

In summary this study has demonstrated the uptake and tissue distribution of aromatic hydrocarbons by English sole from crude oil contaminated sediments and has confirmed the bioavailability of hydrocarbons from sediments into yet another level of the marine food-chain (water column to teleost, Roubal et al. 1978; water column to brown shrimp, Anderson et al. 1974; sediment to soft-shell clam, Vandermeulen and Penrose 1978). In addition, preliminary evidence is presented for long-term physiological and pathological stress following continuous exposure to oil-contaminated sediments.

## Acknowledgments

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# Pathological Changes in a Marine Fish After a 6-Month Exposure to Petroleum[1,2]

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PAYNE, J. F., J. W. KICENIUK, W. R. SQUIRES, AND G. L. FLETCHER. 1978. Pathological changes in a marine fish after a 6-month exposure to petroleum. J. Fish. Res. Board Can. 35: 665–667.

Cunners (*Tautogolabrus adspersus*) were chronically exposed to Venezuelan crude in a flow-through seawater system. The survival rate was >95%. The following parameters were examined: appearance of fish and internal organs from a series of color photographs; fish condition index; hematocrit; somatic indices of liver, heart, spleen, and gonads; eye lens diameter; and plasma chloride. Liver, kidney, heart, spleen, gonad, gill, muscle, and gut tissues were also examined from paraffin sections for pathological changes. There were significant differences in testis somatic index, lens diameter, and plasma chloride between the two fish groups. No histopathological changes were observed.

*Key words:* marine fish, petroleum, chronic exposure, pathological changes

PAYNE, J. F., J. W. KICENIUK, W. R. SQUIRES, AND G. L. FLETCHER. 1978. Pathological changes in a marine fish after a 6-month exposure to petroleum. J. Fish. Res. Board Can. 35: 665–667.

Nous avons soumis des tanches-tautogues (*Tautogolabrus adspersus*) à une exposition chronique à du pétrole brut du Vénézuéla dans un système d'eau salée à écoulement continu. Le taux de survie a été supérieur à 95%. Nous avons examiné les paramètres suivants : l'apparence du poisson et des organes internes d'après une série de photographies en couleurs; le coefficient de condition du poisson; l'hématocrite; les coefficients somatiques du foie, du coeur, de la rate et des gonades; le diamètre du cristallin; et le chlorure plasmatique. Nous avons de plus examiné des coupes histologiques dans de la paraffine de tissus du foie, du rein, du coeur, de la rate, de la gonade, de la branchie, du muscle et du tube digestif en vue d'y détecter des changements pathologiques. Nous avons trouvé des différences significatives dans l'indice somatique du testicule, le diamètre du cristallin et le chlorure plasmatique entre les deux groupes de poissons. Nous n'avons observé aucun changement histopathologique.

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ALTHOUGH intertidal ecosystems appear to be affected by oil spills, there has been little evidence to suggest any adverse effects of petroleum hydrocarbon contamination on subtidal marine life. As a natural component of many marine ecosystems, petroleum should not be generally inimical to life; alternatively those organisms that coexist with oil may have special adaptations. Thus, petroleum like any chemical(s) should be seen as a poison only as a consequence of the quantity with which a host must deal. To further understand dose–effect responses, however, there is a need for studies on the effects of chronic oil exposure on marine life. Anderson's group (1975) has carried out some chronic exposures with invertebrates and the Northwest Fisheries Center in Seattle has recently emphasized long-term studies with fish (Hawkes 1977). We report here a study on growth and pathological changes in a marine fish exposed to a surface slick of Venezuelan crude oil for 6 mo.

## Materials and Methods

Cunners (*Tautogolabrus adspersus*) were collected from a single site in Conception Bay, Newfoundland, and held at the Marine Sciences Research Laboratory, Memorial University. These fish are known to respond to environmental

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TABLE 1. Effects of a 6-mo exposure to petroleum on condition coefficient (*K*), somatic index (SI), eye lens diameter, hematocrit, and plasma chloride in cunners. Values are expressed as means ± SD, except for plasma chloride values, which are means ± SE; sample size is given in parentheses.

| | Control | Oil exposed | Significance (*t*-tests) |
|---|---|---|---|
| *K* (males)[a] | 1.32±0.13 (15) | 1.31±0.20 (17) | $P>0.10$ |
| *K* (females) | 1.25±0.01 (42) | 1.27±0.01 (50) | $P>0.10$ |
| Liver SI[b] | 2.60±0.49 (64) | 2.73±0.55 (66) | $P<0.10$ |
| Heart SI | 0.16±0.07 (56) | 0.16±0.06 (67) | $P>0.10$ |
| Spleen SI | 0.21±0.10 (59) | 0.17±0.09 (55) | $P>0.02$ |
| Ovary SI | 1.53±0.92 (40) | 1.52±0.48 (51) | $P>0.10$ |
| Testis SI | 1.33±0.83 (15) | 0.81±0.30 (18) | $P<0.02$ |
| Eye lens diam (cm) | 0.37±0.03 (35) | 0.41±0.03 (32) | $P<0.01$ |
| Hematocrit (% cells) | 26.36±5.39 (36) | 25.67±4.50 (35) | $P>0.10$ |
| Plasma chloride (mM) | 162±5.88 (24) | 157±5.48 (30) | $P<0.01$ |

[a]Weight/length$^3$.
[b]Grams/100 g body weight.

levels of petroleum by induction of mixed-function oxidases (Payne 1976). For the experiment, fish were contained in two 240-L tanks (1 × 1 × 0.3 m) having a turnover rate of ~2 L of water/min. The proportion of females to males in the experiment was about 3:1. Weathered oil was removed weekly from the surface of the exposure tank and a fresh batch (150 mL) of Venezuelan crude added. No attempt was made to critically quantitate the amount of oil in the water column. The design was to closely simulate a "water-soluble" exposure. The flow rate was 2–2.5 L of water/min; thus each 150-mL titer was "extracted" with approximately 20 000 L of water. Hydrocarbon fluorescence (Keizer and Gordon 1973) was often undetectable in water extracts after a 1-d exposure. During the warmer summer temperatures, however, when fish were actively feeding, small oil particulates often appeared in the water column. We have observed in acute oil exposures that fish will sometimes ingest large amounts of particulate oil prior to death while lying in a vertical position and gaping at the surface. No such gaping behavior was noted in this study and there was no indication of particulate oil in the guts of fish when killed. Control and experimental fish were fed weekly an equivalent amount of herring or capelin. Food was added in excess and removed after 1–2 d. There were no obvious differences in activity or feeding rates. Fish were exposed from May to November 1976; water temperatures ranged from about −1.4°C during the winter (January–April) to 14°C or more in summer (July–October). Cunners are torpid during the winter and only feed actively at warmer summer temperatures (Naidu 1966).

At the termination of the experiment the following parameters were examined: gross appearance of external tissues and internal organs from a series of color photographs; growth factors including fish condition coefficient ($K$), where $K$ = length/weight$^3$, and organ somatic indices (SI), where SI = (wet weight organ/weight of fish) × 100, for liver, heart, spleen, and gonads; eye lens diameter; hematocrit; and serum chloride. Blood samples were obtained by cardiac puncture in sodium heparin; plasma chlorides were determined by titration (Radiometer Copenhagen Model CMT 10). Paraffin sections of liver, heart, spleen, gonad, gill, kidney, muscle, and gut tissues were also examined for histopathological changes; 16 fish from each group were examined. Tissues were fixed in Bouin's, Dietrich, or phosphate (Karnovsky's) buffered 1% formalin. Various stains were used including Azure B, hematoxylin–eosin, Mallory triple, or Heidenhain's Azan triple. The Heidenhain Azan method is time consuming but was generally preferred, since it provided more staining detail in some tissues.

## Results and Discussion

### Survival and Growth Characteristics

Survival rate of fish in control and experimental tanks was >95%. The series of photographs revealed no differences in the appearance of external tissues or internal organs between fish groups. Discoloration of liver (Cardwell 1973; Waluga 1966) and kidney (Waluga 1966) has been noted in acute exposures of fish to phenols or refined oil. We have also noted liver and bile discoloration in fish acutely exposed to Venezuelan crude at concentrations normally lethal after a 1–2-wk exposure.

Condition indices were not significantly different ($P > 0.02$) between fish groups nor were somatic indices for liver, heart, spleen, and ovaries (Table 1). There was a difference in the spleen somatic index between fish groups (approaching the 0.02 probability level) but a significant difference ($P < 0.02$) was noted only for testes, which appeared to be reduced in weight in the oil-exposed fish.

Because a uniform population of fish is being analyzed in these studies, any significant change in growth or "body wastage" between groups would under normal circumstances be reflected in a change of condition coefficient. In recent years organ somatic indices have been routinely examined in toxicological studies with mammals. Either growth (permanent addition of differentiated tissue) or energy storage change may be important in assessing relative organ weight changes. Decreased growth and liver glycogen were observed in trout fed a diet containing 1% crude petroleum for 75 d (Hawkes 1977). Acute oil exposures can decrease liver energy reserves in fish (Sabo et al. 1975) but this effect may be not unlike situations of increased activity or short-term food deprivation. A decrease in testis weight was the only significant somatic index change noted in this study. Stress is known to affect testis size in mammals and polychlorinated biphenyl has been reported to have such an effect in trout (Freeman and Idler 1975). However, reproduction was not affected in trout fed a diet containing 1% crude petroleum for a year (Hodgins et al. 1977).

One of the most interesting effects found in this study was the increase in eye lens diameter in fish exposed to petroleum (Table 1). Hawkes (1977) originally observed this effect in trout fed a diet containing 1% crude for several months. The present study, using a much lower dose of petroleum, suggests that such an effect may occur in the environment near chronic hydrocarbon exposure sites, such as refinery outfalls.

### Blood and Ionic Homeostasis

There were no differences in hematocrit values between fish groups but plasma chloride was significantly decreased in the oil-exposed fish (Table 1). Chloride decrease could be due to increased hydration of the oil-treated fish, which may account for the increased eye lens size. Crude oil can interfere with rates of $Na^+$ and water transport across the intestinal mucosa in birds (Crocker et al. 1975).

### Histopathology

There were no structural or histochemical changes in various tissues from the two fish groups. The tissues examined included liver, kidney, heart, spleen, gonad, gill, muscle, and gut. Structural lesions have been reported in various fish tissues after acute or chronic hydrocarbon exposure. Acute exposures to petroleum or petroleum-type compounds caused gill epithelial sloughing and mucosal cell changes in various species (Blanton and Robinson 1973; Gardner 1975; Hawkes 1977). In a study of flounder skin mucous glands, Hawkes (1977) noted that results were inconclusive, since there was a large variability in the number of glands and the number that had discharged their contents. We have noted the same effect in trout with acute Venezuelan crude oil exposures (Payne unpublished data). Rainbow trout fed high concentrations of oil developed liver lesions characterized by an increase of sinusoid collagen (Hawkes 1977). No such lesions were noted in this study. Spleen discoloration changes which may be related to red blood cell destruction have been noted in acute exposures of salmon to No. 2 fuel oil (Cardwell 1973). No changes were noted in external color or histology of the spleen between the two cunner groups, but it should be noted that the decrease in the spleen mean somatic index in the oil-exposed cunners approached the 0.05% probability level of significance.

In conclusion, the sublethal effects of a chronic oil exposure to cunners included a slight reduction in testis weight, a reduction in plasma chloride, and an increase in eye lens diameter. Of the three effects, eye lens change is a tangible biological response and should be assessed in environmental samples of fish.

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# Influence of Environmental Contamination on Cytochrome P-450 Mixed-Function Oxygenases in Fish: Implications for Recovery in the Wild Harbor Marsh[1]

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STEGEMAN, J. J. 1978. Influence of environmental contamination on cytochrome P-450 mixed-function oxygenases in fish: implications for recovery in the Wild Harbor Marsh. J. Fish. Res. Board Can. 35: 668–674.

Levels of hepatic cytochrome P-450 and the catalytic functions benzo[*a*]pyrene hydroxylase and aminopyrine demethylase were significantly greater in populations of mummichog, *Fundulus heteroclitus*, sampled from contaminated marshes than in those fish from nearby reference marshes. Aminopyrene demethylase, but not benzo[*a*]pyrene hydroxylase, was also elevated in fish from one of the reference marshes. The levels of cytochrome P-450 and benzo[*a*]pyrene hydroxylase were highest in fish from the Wild Harbor Marsh, site of the 1969 *Florida* spill. A correlation between size and benzo[*a*]pyrene hydroxylase activity was identified in some of the populations studied, although influence by this factor did not mask the population differences. The results are consistent with the hypothesis that elevated levels of mixed-function oxygenase activity in fish from Wild Harbor are the result of oil spilled 8 yr ago, and that recovery of this marsh is not yet complete.

*Key words:* mixed-function oxygenases, benzo[*a*]pyrene hydroxylase, aminopyrine demethylase, cytochrome P-450, *Fundulus heteroclitus*, environmental contamination, oil spill recovery, petroleum

STEGEMAN, J. J. 1978. Influence of environmental contamination on cytochrome P-450 mixed-function oxygenases in fish: implications for recovery in the Wild Harbor Marsh. J. Fish. Res. Board Can. 35: 668–674.

Les niveaux de cytochrome P-450 hépatique et les fonctions catalytiques benzo[a]pyrène hydroxylase et aminopyrène déméthylase sont nettement plus élevés dans les populations de le choquemort, *Fundulus heteroclitus*, échantillonnés dans des marais contaminés par comparaison avec ceux de poissons provenant de marais témoins. Dans un des marais témoins, l'aminopyrène déméthylase est élevée, mais la benzo[*a*]pyrène hydroxylase ne l'est pas. Les niveaux maximums de cytochrome P-450 et de benzo[*a*]pyrène hydroxylase se rencontrent dans les poissons du marais de Wild Harbor, site du déversement du *Florida* de 1969. Dans quelques-unes des populations étudiées, nous avons établi une corrélation entre la taille et l'activité du benzo[*a*]pyrène hydroxylase, bien que l'influence de ce facteur ne masque pas les différences entre les populations. Ces résultats sont conformes à l'hypothèse que les niveaux élevés d'activité des oxygénases à fonction mixte chez les poissons de Wild Harbor sont le résultat d'un déversement de pétrole qui se produisit il y a 8 ans, et que ce marais ne s'est pas encore rétabli.

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CYTOCHROME P-450 is a generic name given to a family of hemoproteins which exhibit Soret-absorption maxima at or near 450 nm in the CO-bound reduced state and which are involved in foreign-compound metabolism. Microsomal cytochrome P-450 dependent mixed-function oxygenase systems are perhaps ubiquitous in vertebrate species, including fishes. Fish hepatic microsomal cytochrome P-450 possesses optical and magnetic properties qualitatively similar to the better known cytochromes P-450 from mammals; i.e. reduced, CO-ligated fish cytochrome P-450 exhibits a Soret-absorption maximum near 450 nm, and EPR (electron paramagnetic resonance) spectra of fish cytochrome P-450 exhibit low-spin $g$ values near 2.4, 2.24, and 1.9 (Chevion et al. 1977). As with mammalian systems, biotransformation of a great variety of substrates is catalyzed by cytochrome P-450 systems in fish, ranging from endogenous compounds such as steroid hormones to foreign compounds such as aromatic hydrocarbons

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(Inano et al. 1976; Diamond and Clark 1970).

Treatment of fish with selected compounds such as 5,6-benzoflavone or the polynuclear aromatic hydrocarbon 3-methylcholanthrene will, as in mammals, induce increased levels of hepatic benzo[*a*]pyrene hydroxylase activity (Bend et al. 1973; Chevion et al. 1977). Treatment with petroleum can produce a similar induction (Payne and Penrose 1975; Vandermuelen et al. 1978) and it is this feature of cytochrome P-450 systems in fish that lends itself to potential use as an indicator of effects of environmental contamination by petroleum hydrocarbons and other organic pollutants.

This report is concerned with the hepatic mixed-function oxygenase activities of benzo[*a*]pyrene hydroxylase and aminopyrine demethylase in populations of *Fundulus heteroclitus* (killifish or mummichog) from several sites, including the Wild Harbor Marsh, an area severely contaminated by a highly aromatic No. 2 fuel oil spilled from the *Florida* in 1969. *Fundulus* were selected for this study as they are common in marshes and estuaries where they reside in a fairly restricted area (Lotrich 1975) and thus reliably reflect the environment from which they are sampled. The results are considered generally as they relate to the potential use of mixed-function oxygenases as environmental indicators, and more specifically as they relate to the extent of biological recovery evident in Wild Harbor Marsh 8 yr after the oil spill.

## Materials and Methods

*Fundulus* were sampled by seining in each of four marshes on Cape Cod, Mass.: Monomoscoy, a small marsh on Monomoscoy Island bordering the East River in Waquoit Bay; Little Sippewissett, Great Sippewissett, and Wild Harbor, each bordering Buzzards Bay. Wild Harbor was chosen as a petroleum-contaminated site. Great Sippewissett was also selected as a contaminated site, a portion of this marsh having been experimentally treated for the past 6 yr with sludge fertilizer containing high levels of chlorinated and petroleum-derived hydrocarbons (Krebs et al. 1974). Monomoscoy and Little Sippewissett were chosen as reference marshes, with no known sources of organic contamination.

Fish were collected from approximately the same locations each time in Wild Harbor and Little Sippewissett, and exactly the same locations in Monomoscoy and Great Sippewissett. The animals were returned to the laboratory alive and in most cases maintained in their own water until killing and analysis which took place within 24 hr. At each sampling fish were grouped according to sex and size; i.e. a given group contained fish that were all ±10% of the group mean body weight. In all cases groups were analyzed at least in pairs.

Whole livers from fish in each group were pooled (excluding any individuals parasitized or with punctured gallbladders), homogenized 20% wt/vol in 0.065 M phosphate buffer, pH 7.3, containing 3 mM $MgCl_2$ and 1.15% KCl, and a 9000 × *g* supernatant (postmitochondrial supernatant; PMS) fraction prepared. PMS preparations were diluted to 2.0–3.0 mg protein/mL and cytochrome P-450 was analyzed optically in CO-bound, dithionite-reduced samples vs. CO-bound reference, with a Cary 118C recording spectrophotometer. Cytochrome content was determined using an $OD_{450-490}$ extinction coefficient of 91 $mM^{-1} \cdot cm^{-1}$ (Omura and Sato 1964).

Benzo[*a*]pyrene hydroxylase was determined in a 1.0-mL reaction mixture containing 5 μmol NADP (Sigma), 15 μmol G-6-P (Sigma), 2 U G6PD (Sigma), $3 \times 10^{-3}$ M $MgCl_2$, and 1.5–2.0 mg PMS protein, in 0.1 M phosphate. The pH of the reaction mixture was 7.0. The generating system was allowed to preincubate for 15 min. The reaction was initiated by addition of 15.6 μg of benzo[*a*]pyrene (Aldrich) in 40 μL of methanol and incubated in a shaking water bath at 29°C for 30 min. These conditions were determined to be optimal in this laboratory. The reaction was stopped and the hydroxylated product was extracted and assayed fluorometrically in a procedure similar to that of Nebert and Gelboin (1968), with authentic 3-OH-benzo[*a*]pyrene as a reference standard.

Aminopyrine demethylase was assayed in a 1.5-mL reaction mixture containing an NADPH generating system and PMS protein as above, in 0.166 M HEPES (Sigma), pH 7.6. The reaction was initiated by adding twice recrystallized aminopyrine, incubated at 29°C for 15 min, and stopped by adding 0.5 mL $ZnSO_4$ (25% saturated) and 0.5 mL BaOH (saturated). Formaldehyde was determined according to Nash (1953) as modified by Cochin and Axelrod (1959). Formaldehyde standards were incubated and treated as samples. Controls for benzo[*a*]pyrene hydroxylase and aminopyrine demethylase consisted of reactions carried out without the NADPH generating system.

Protein was determined according to Lowry et al. (1951), with bovine serum albumin as a standard. All analyses were performed in duplicate, and data were analyzed by regression analysis and standard *t*-tests performed on pooled variance estimates (Sokal and Rolf 1969).

## Results

As indicated in Table 1, fish from each marsh were sampled in 1976 and 1977, and in this report data from the 2 yr are considered together. The numbers of males and females were equivalent and the range of sizes sampled was more than fivefold in each marsh, yet the mean size of animals in all groups did not differ significantly from marsh to marsh. Although animals were grouped by sex as well as size, the data presented are means for all groups within a marsh unless otherwise indicated.

The PMS protein content per gram of liver appeared to be somewhat lower in the fish from Little Sippewissett than in the other marshes (Table 1). However, the variance was rather high in all cases and the differences between marshes were not significant.

### Benzo[*a*]pyrene Hydroxylase

With Monomoscoy as the primary reference, benzo[*a*]pyrene hydroxylase activity per milligram of PMS protein (Table 2) exhibited a trend to greater activity in fish from contaminated marshes, yet the differences were not significant, perhaps a feature related to the high variance in PMS protein content. When the data were normalized to liver weight, the

TABLE 1. Sites, dates, and sizes sampled, and hepatic PMS protein in *Fundulus heteroclitus*.

| Marsh | *N* fish | *N* groups (♂–♀) | Dates sampled | $\bar{x}$ size (± SD) (g) | Size range (g) | mg PMS protein / g liver |
|---|---|---|---|---|---|---|
| Monomoscoy (reference) | 64 | 6 (3–3) | July 1976<br>Aug. 1977 | 7.61 (±3.14) | 2.60–13.23 | 93.2 (±16.5) |
| Little Sippewissett (reference) | 49 | 8 (4–4) | July, Oct. 1976<br>July 1977 | 8.55 (±6.48) | 2.78–25.57 | 81.6 (±12.7) |
| Great Sippewissett (contaminated) | 53 | 7 (4–3) | July 1976<br>July, Aug. 1977 | 6.26 (±2.79) | 1.90–14.19 | 92.5 (±18.7) |
| Wild Harbor (contaminated) | 149 | 23 (11–12) | July, Oct. 1976<br>June, July,<br>Aug. 1977 | 8.92 (±4.32) | 2.94–17.74 | 97.1 (±19.0) |

TABLE 2. Hepatic benzo[*a*]pyrene hydroxylase and aminopyrine demethylase activity in *F. heteroclitus*.

| Marsh | Units / mg PMS protein | Units / g liver ($\times 10^3$) | Units / g body weight |
|---|---|---|---|
| | *Benzo[a]pyrene hydroxylase*[a] | | |
| Monomoscoy | 89.2 (±28.4)[e] | 7.58 (±1.72) | 143.3 (±36.0) |
| Little Sippewissett | 73.8 (±32.9) | 5.72 (±1.43) | 178.3 (±63.6) |
| Great Sippewissett | 101.1 (±19.2) | 9.00 (±1.00)[d] | 246.7 (±40.7)[i] |
| Wild Harbor | 108.8 (±31.5) | 10.15 (±2.29)[f] | 278.8 (±70.9)[j] |
| | *Aminopyrine demethylase*[b] | | |
| Monomoscoy | 16.4 (±4.8) | 1.43 (±0.62) | 27.8 (±13.8) |
| Little Sippewissett | 44.6 (±4.2)[j] | 4.16 (±0.24)[j] | 105.7 (±11.0)[j] |
| Great Sippewissett | 25.7 (±6.9)[d] | 2.12 (±0.15)[e] | 57.0 (±7.5)[h] |
| Wild Harbor | 36.4 (±11.4)[g] | 3.72 (±0.77)[i] | 95.0 (±41.8)[g] |

[a]Units = picomoles 3-OH-benzo[*a*]pyrene equivalents per minute.
[b]Units = nanomoles HCHO produced per hour.
[c]Values in parentheses are ± standard deviation.
[d–j]Significantly different from the primary reference (Monomoscoy) at $0.10 > P > 0.05$(d); $P \leq 0.05$(e); $0.02 > P > 0.01$(f); $P \leq 0.01$(g); $P \leq 0.005$(h); $P \leq 0.001$(i); $P \leq 0.0001$(j).

extent of difference between fish from contaminated and uncontaminated marshes remained about the same, although a degree of significance was acquired. However, when normalized to body weight the activity in fish from contaminated marshes was almost twice that in fish from Monomoscoy and the differences were very highly significant. Fish from the two uncontaminated marshes were essentially identical.

The results expressed in Table 2 are means obtained from animals grouped in a wide range of size-classes. Plotting the activity of benzo[*a*]pyrene hydroxylase per gram of body weight in these groups against size discloses a relationship between the two. This relationship is depicted in Fig. 1 for fish from Monomoscoy. Similar results were obtained for fish from a contaminated marsh (Fig. 2), although in this instance the intercept was much greater, while the slope remained essentially the same. Thus the differences in benzo[*a*]pyrene hydroxylase activity between fish from these two marshes become even more pronounced when the data are expressed this way, yet the nature of the relationship between size and activity appears to remain the same in fish from both marshes. The relationship appearing in fish from these two marshes was consistently observed in each pair of groups sampled. The high correlation coefficients and the fact that the points represent fish sampled in different years make this relationship appear even more striking.

In contrast, fish from Wild Harbor Marsh did not exhibit the same relationship between size and activity ($a = 251.8$, $b = 3.16$, $r = 0.189$, $P = 0.200$). There was a high degree of randomness associated with the data here and even in pairs of groups taken from the same seine no such relationship was evident. However, the distinction between Monomoscoy and Wild Harbor fish remains quite obvious and if anything the randomness appearing in Wild Harbor suggests the influence of a disturbing factor, possibly related to the oil, not present in Monomoscoy or even in Great Sippewissett.

Most groups of fish in Little Sippewissett displayed a size–activity relationship like the one seen in Monomoscoy fish, with a similar slope and intercept ($a =$

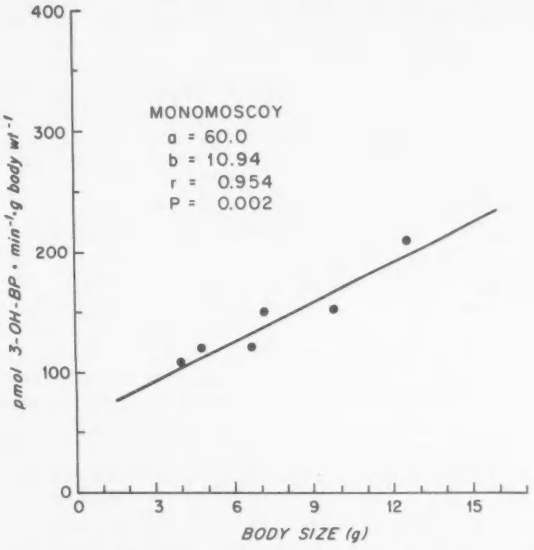


FIG. 1. Relationship between hepatic benzo[*a*]pyrene hydroxylase activity and size in *Fundulus heteroclitus* from Monomoscoy. *a* = Intercept; *b* = slope; *r* = correlation coefficient; *P* = significance of the correlation. The assumption of linearity to the intercept is not necessarily valid.

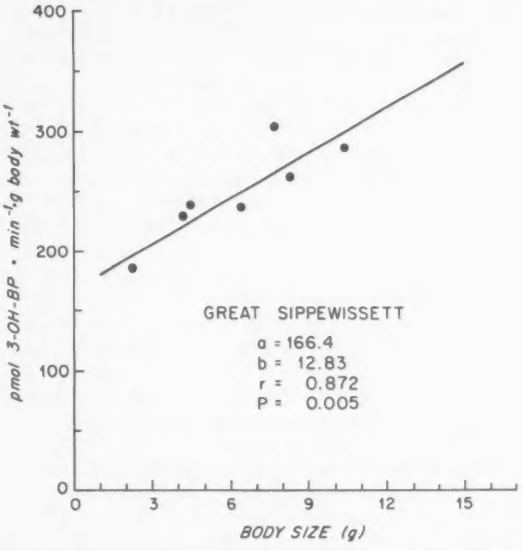


FIG. 2. Relationship between hepatic benzo[*a*]pyrene hydroxylase activity and size in *F. heteroclitus* from Great Sippewissett. *a* = Intercept; *b* = slope; *r* = correlation coefficient; *P* = significance of the correlation.

74.3, $b = 8.54$, $r = 0.954$, $P = 0.004$). There were, however, other groups in this marsh that did not fit this pattern, for reasons that are not apparent.

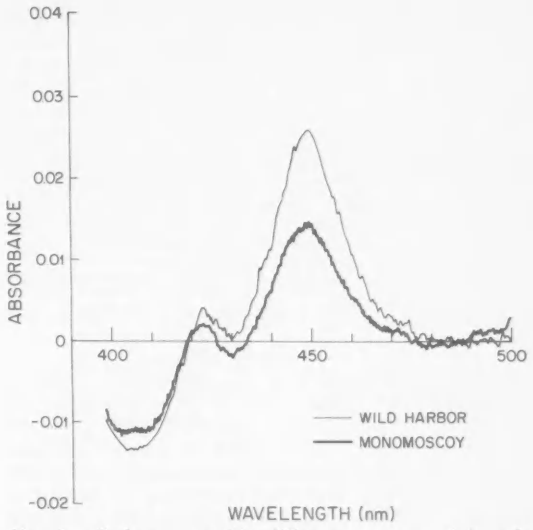


FIG. 3. Carbon monoxide difference spectra of hepatic PMS prepared from *F. heteroclitus*. Fish used in these determinations were adult males sampled after the spawning season. Spectra were determined in 0.1 M phosphate buffer, pH 7.0. Protein concentration in these determinations was 2.75 mg/mL in the Wild Harbor sample and 2.87 mg/mL in the Monomoscoy sample.

There were differences in mixed-function oxygenase activity linked to sex differences and seasonal variation in addition to size, similar to those differences described for other species (Stegeman 1977). These factors did not, however, affect the differences seen between populations.

### AMINOPYRINE DEMETHYLASE

The activity of hepatic aminopyrine demethylase was significantly greater in Wild Harbor and Great Sippewissett fish than in those from the primary reference marsh, Monomoscoy. This was true whether the data were normalized to PMS protein, liver weight, or body weight (Table 2). Unlike the situation observed with benzo[*a*]pyrene hydroxylase, however, there was also significantly greater aminopyrine demethylase activity in the fish from Little Sippewissett. In fact, fish from this marsh possessed the greatest demethylase activity. There was no evident relationship between body size and the activity of aminopyrine demethylase in fish from any marsh.

### CYTOCHROME P-450

The optical properties of the cytochrome P-450 complement in the PMS fractions displayed qualitative similarity in all groups of fish, with a peak absorption in the Soret region at 450 nm (e.g., Fig. 3). As suggested in Table 3, however, there were quantitative dif-

TABLE 3. Hepatic cytochrome P-450 content in *F. heteroclitus*.

| Marsh | P-450[a] mg PMS protein | P-450 g liver | P-450 g body weight |
|---|---|---|---|
| Monomoscoy | 0.055 (±0.006)[b] | 5.11 (±1.19) | 0.099 (±0.043) |
| Little Sippewissett | 0.064 (±0.016) | 5.06 (±0.63) | 0.156 (±0.032)[d] |
| Great Sippewissett | 0.066 (±0.016) | 5.94 (±1.46) | 0.164 (±0.054)[e] |
| Wild Harbor | 0.072 (±0.016)[d] | 6.70 (±1.25)[e] | 0.189 (±0.067)[f] |

[a]Nanomoles of cytochrome P-450.
[b]± Standard deviation.
[c–f]Significantly different from the primary reference (Monomoscoy) at $P \leq 0.04$(c); $P \leq 0.02$(d); $P \leq 0.01$(e); $P \leq 0.005$(f).

ferences in cytochrome P-450 among fish from the various marshes. Only in fish from Wild Harbor was the content of hepatic cytochrome P-450 per milligram of PMS protein or normalized to liver weight significantly greater than in fish from Monomoscoy. However, when normalized to body weight, the content of hepatic cytochrome P-450 was significantly greater in fish from Great Sippewissett and Little Sippewissett, in addition to Wild Harbor.

Based on a rough estimate of turnover number (i.e. activity per unit P-450), it appears that the cytochrome P-450 complement in Wild Harbor fish, for example, is no more efficient at catalyzing benzo[*a*]pyrene hydroxylation than is the cytochrome complement in Monomoscoy fish. Thus while there is a greater benzo[*a*]pyrene hydroxylase activity in fish from the contaminated marshes, it may be a feature of the higher levels of cytochrome P-450. On the other hand, the higher aminopyrine demethylase activities in Wild Harbor and Little Sippewissett fish appear to be associated not just with increased cytochrome P-450 content but also with a cytochrome complement that is more efficient at catalyzing this reaction.

## Discussion

The results presented here describe elevated levels of benzo[*a*]pyrene hydroxylase, aminopyrine demethylase, and cytochrome P-450 in *Fundulus heteroclitus* sampled from areas with known sources of organic contaminants, in comparison with fish from ostensibly clean areas. Previous reports dealing with putative induction of fish mixed-function oxygenases in the environment have usually involved a single sampling of animals at a given site, with analysis individually or pooled (Burns 1976; Payne 1976; Pederson et al. 1976; Stegeman and Sabo 1976). In this study there was repeated sampling, extending over a period of years, and the differences observed were highly significant.

The appearance of higher benzo[*a*]pyrene hydroxylase activity is consistent with other recent reports suggesting that levels of this mixed-function oxygenase activity can be linked to environmental contamination (Payne 1976; Pederson et al. 1976; Stegeman and Sabo 1976). This may result from induction of benzo[*a*]pyrene hydroxylase by environmental contaminants similar to that observed to occur upon treatment of some fish species with known "Type II" inducers (Bend et al. 1973; Chevion et al. 1977; Pederson et al. 1976) or with the complex mixtures of compounds that make up petroleum (Payne and Penrose 1975). The areas sampled in Wild Harbor and Great Sippewissett are characterized by high levels of aromatic and, in the case of Great Sippewissett, chlorinated hydrocarbons which could well be acting as such inducers. However, the observation that differences in benzo[*a*]pyrene hydroxylase were most significant when activity was normalized to body weight implies that increased liver size may also be important in conferring an increased capacity for this, and possibly other hydrocarbon biotransformation activities, on these animals.

Aminopyrine demethylase activity, like benzo[*a*]-pyrene hydroxylase, was found to be higher in fish from the contaminated marshes. Interestingly, the demethylase activity was also very high in animals from Little Sippewissett, one of the marshes selected as being uncontaminated and where there was low benzo[*a*]pyrene hydroxylase activity. The reason for this discrepancy is unknown, although the possibility clearly exists that some unsuspected contaminant(s) may be inducing this mixed-function oxygenase activity in Little Sippewissett fish. PCB's, as an example, have been successfully used to induce aminopyrine demethylase in at least one species of fish (Hill et al. 1976), and the possibility of chlorinated hydrocarbons, or other compounds, entering the marsh from nearby homesites cannot be excluded. Similar circumstances may be working to produce the apparent scatter in benzo[*a*]pyrene hydroxylase in these fish.

It is well known that benzo[*a*]pyrene hydroxylase and *N*-demethylase activities can be differentially induced by selected compounds in mammals, and that this involves different cytochromes P-450 (Lu et al. 1972). Furthermore, it has been found in at least one species of fish that the "Type II" inducer 3-methylcholanthrene will induce benzo[*a*]pyrene hydroxylase activity but not benzphetamine demethylase (Bend et al. 1977). Thus, a lack of correlation between benzo[*a*]pyrene hydrox-

ylase and aminopyrine demethylase activities in *Fundulus* from Little Sippewissett need not be surprising, and suggests the possible involvement of multiple cytochromes P-450 in this species.

A higher cytochrome P-450 content was observed in conjunction with either higher hydroxylase or demethylase activities. The differences seen here were on the same order of magnitude as the differences in cytochrome P-450 content found by Burns (1976) in *Fundulus* sampled from Wild Harbor, Great Sippewissett, and selected uncontaminated marshes, although in that report significance was not determined. If the higher activities are due to induction, then this result is unusual for it stands in sharp contrast to reports describing induction of mixed-function oxygenases in fish, particularly of benzo[*a*]pyrene hydroxylase, without any apparent increase in cytochrome P-450 content (Bend et al. 1977; Chevion et al. 1977).

Aside from the increased levels, there were no differences seen in the optical properties of cytochrome P-450, a situation similar to that described by Burns (1976) for these fish. Induction of benzo[*a*]pyrene hydroxylase by "Type II" inducers in many mammalian species is accompanied by a shift in the absorption maximum of the cytochrome P-450 complement, due to an increase of a cytochrome with different spectral properties (Alvares et al. 1967). While this was initially thought not to be the case in fish (Chevion et al. 1977), the appearance of different spectral forms of cytochrome P-450 upon induction has been determined for little skate, *Raja erinacea*, although this was only after resolution of the cytochromes (Elmamlouk et al. 1977). A similar situation may occur in other cases, including the present one.

The results presented here support the contention that fish mixed-function oxygenases may in some circumstances be used as indicators of environmental contamination by foreign compounds including, but in no way restricted to, some fraction of petroleum. The results also suggest that it may not be sufficient to use a single catalytic function as an indicator. Unknown factors, such as are possibly at work in the Little Sippewissett Marsh or in trout sampled by Pederson et al. (1976), may influence interpretation of results. This includes the possibility that contamination may eventually produce lesions resulting in decreased mixed-function oxygenase activities (Ahokas et al. 1976). Clearly work is required to further clarify those circumstances, probably limited, under which use of mixed-function oxygenases as indicators may be validly interpreted.

Nevertheless, in the case of Wild Harbor, the comparatively greater levels of benzo[*a*]pyrene hydroxylase, aminopyrine demethylase, aldrin epoxidase (Burns 1976), and cytochrome P-450 in *Fundulus* from this marsh strongly suggest that a relationship exists between these higher mixed-function oxygenase activities and the fuel-oil hydrocarbons and that these fish still show effects 8 yr after the spill. This is in agreement with the demonstration that substantial amounts of aromatic hydrocarbons remain in Wild Harbor (Teal et al. 1978) and that other members of the biota there are still being affected (Krebs and Burns 1977). It is not possible, however, to distinguish between whether the high mixed-function oxygenase activities observed currently result from a contemporary event, possibly induction, or from a selection for this condition. Similarly, we do not know what fractions of petroleum or petroleum products may be involved, nor whether apparent induction identified in vitro indicates enhanced biotransformation and elimination of hydrocarbons in vivo.

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# Oiled Seabird Rescue and Conservation[1]

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CLARK, R. B. 1978. Oiled seabird rescue and conservation. J. Fish. Res. Board Can. 35: 675–678.

Attempts were made to clean and rehabilitate nearly 8000 oiled birds (mainly auks) following the wreck of the *Torrey Canyon*. This was an almost total failure and a Research Unit on the Rehabilitation of Oiled Seabirds was established in Newcastle upon Tyne to devise effective treatment methods. This has involved a study of the mechanism of water-repellency in seabird plumage and led to the development of cheap, effective cleaning methods and full recovery of the birds within 2–3 wk of capture. These methods have been used successfully on small numbers of birds by amateurs and also operationally on larger numbers by the Research Unit. There have been only few ringing returns but at least some of the cleaned birds became reintegrated with the breeding populations after release. Even with appropriate organization and facilities it is doubtful whether the rescue and treatment of oiled birds can make a material contribution to seabird conservation, but there is often strong public pressure for the treatment of oiled birds and the development of suitable techniques makes this possible.

*Key words:* oiled seabird rescue, conservation, water-repellent plumage

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On a essayé de nettoyer et réhabiliter près de 8 000 oiseaux (des pingouins et grande partie) recouverts de pétrole à la suite du naufrage du *Torrey Canyon*. Ce fut un échec presque total, et l'on mit sur pied à Newcastle upon Tyne une unité de recherche sur la réhabilitation des oiseaux de mer affectés par le pétrole, dans le but de découvrir des méthodes efficaces de traitement. On a étudié le mécanisme d'imperméabilité du plumage des oiseaux de mer. Les résultats de l'étude ont été la découverte de méthodes efficaces et peu dispendieuses de nettoyage et rétablissement complet des oiseaux en dedans de 2 à 3 sem après la capture. Ces méthodes ont été appliquées avec succès sur de petits nombres d'oiseaux par des amateurs et aussi sur de grands nombres au cours des opérations de l'unité de recherche. Les retours d'oiseaux bagués, quelque rares qu'ils soient, indiquent au moins que les oiseaux nettoyés se sont réintégrés aux populations reproductrices après mise en liberté. Il est douteux que le traitement d'oiseaux recouverts de pétrole, même avec une organisation et des installations appropriées, contribue matériellement à la conservation des oiseaux de mer. Mais le public exerce souvent de fortes pressions pour que l'on traite les oiseaux affectés par le pétrole, traitement rendu possible par des techniques appropriées.

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FOLLOWING the wreck of the *Torrey Canyon* in March 1967, some 8000 oiled seabirds, mainly guillemot (*Uria aalge*) and razorbill (*Alca torda*), were rescued from beaches in southwest England and distributed to individuals and organizations over a wide area for cleaning and rehabilitation. The operation was an almost total failure. One or two birds were restored to health and survived a number of years in captivity, but it is nearly certain that none were restored to the natural population. The overwhelming majority died as a direct result of oiling or, it must be said, as a consequence of the treatment they received during cleaning and the attempted rehabilitation.

As a result of this disastrous experience, in November 1967 the Advisory Committee on Oil Pollution of the Sea established a Research Unit on the Rehabilitation of Oiled Seabirds in the University of Newcastle upon Tyne. Funds for this were initially provided by national and international animal welfare organizations and the U.K. Institute of Petroleum, but from 1970 until the Unit closed in September 1975, it was supported through the Advisory Committee by donations from a number of oil companies.

Although a wide variety of water birds are at risk from oil pollution, the most important casualties in the Northern Hemisphere are auks and some diving sea ducks. Because they are gregarious and spend much of

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their time on the sea, and dive rather than fly up when disturbed, these species are particularly exposed to contamination by floating oil and if contaminated are likely to be heavily coated with oil. Furthermore, because of their extremely low reproductive rate they are unable to restore losses from the population except over a very long time. In British waters auks are the chief casualties of oil pollution and their numbers have been declining in many British colonies during this century (though this is not entirely due to oil pollution). They are also the most difficult birds to keep healthy in captivity. The Research Unit therefore focussed its attention on these birds, and principally on guillemots. If suitable treatment methods could be devised for these, other species would present no problem.

## Research Programs of the Unit

One of the first tasks of the Unit was to find out what was already known about the effect of petroleum oils on seabirds and also to try and distil the experience of the numerous people who had tried to clean and treat oiled seabirds (Clark 1969; Clark and Kennedy 1968, 1971). From these enquiries it became clear that the mortality of oiled birds fell into two categories: the effects of oiling itself (hypothermia, drowning, pneumonia, enteritis, starvation) and what may be described as diseases of captivity (aspergillosis, infective arthritis, enteritis) while the birds were being treated. The longer the birds remained under treatment, the less their chance of surviving and, of course, even for the very few that did survive, the longer their period in captivity, the less their chance of being successfully reintegrated in a wild population.

It had never proved possible to clean oiled birds and return them to sea quickly because it was universal experience that whatever the method of cleaning, the plumage had lost its water-repellent property and if placed on the water in this condition, the birds rapidly became waterlogged, losing their buoyancy and thermal insulation. This condition persisted until the birds had replaced their plumage at the next molt and often even then it was not fully water-repellent. Rehabilitation of oiled seabirds could never succeed unless a treatment could be devised that restored the water-repellent properties of the feathers after the contaminating oil had been removed.

Very little was known about the water-repellency of feathers at that time. There were two schools of thought, though with little experimental evidence for either: one, that it was due to preen-gland waxes which are spread over the feathers during preening, the other that it was due to the physical structure of the feathers. Research in the Unit by Kennedy (1970a, b, 1971, 1972) established that the latter was of prime importance. The system of interlocking barbs of feathers provides a strongly water-repellent surface, similar to that in some textiles, and the system is arranged in such a way that there is a gradient in water-repellency from the base to the tip of the feather so that a drop of water placed on it tends to run off towards the tip. A monolayer of preen-gland wax on the feather also resists wetting, but it is subservient to feather structure.

Although Larsson and Odham (1970) developed a synthetic preen-gland wax, Larodan 127, which they claimed restored the water-repellent properties of seabird plumage after cleaning, we thought it unlikely that these waxes were responsible for the wettable plumage of cleaned birds. Samples were examined of many oiled birds that were living in captivity or had died after cleaning and in all of them the feathers were contaminated either by residual oil, by traces of surface active cleaning agents, or by fecal material. It was possible to clean oiled feathers, leaving them fully water-repellent, by using organic solvents in an ultrasonic bath and it has proved almost impossible to remove the monolayer of wax from the feathers by any normal cleaning method. We therefore disregarded preen-gland waxes in subsequent investigations.

It was, of course, not practicable to clean living birds in ultrasonic baths, but with the development of a low-toxicity organic solvent by ICI, Arklone P, we were able to clean oiled birds successfully. The solvent left no residues on the feathers and after drying the plumage with a hair drier, the birds were able to swim without becoming wet (Clark and Gregory 1971). Organic solvents suffer several disadvantages that make them unsuitable for general use, particularly by amateurs. They are inflammable and Arklone P vapor is strongly anesthetic. They are not readily available in an emergency and would therefore need to be stockpiled; furthermore they are expensive. Although Naviaux (1971) used organic solvents to clean oiled birds in California, we extended our search to other cleaning agents.

The material that satisfied the criteria of effectiveness, safety, and ready availability was domestic washing-up liquid detergent (RUROS 1972a). Used as a 1% solution in water at 40°C, it removed oil effectively and unlike many other detergents, it does not adsorb strongly onto feathers and can be rinsed off completely without much difficulty. An additional advantage of using a hot solution is that not only is the detergent more effective at this temperature, but the birds are not exposed to thermal shock. Once the birds have been dried, they are able to take to the water without the plumage wetting.

Once the plumage has been adequately cleaned, sea ducks and auks spend much of their time on the water preening. This evidently removes any final traces of contaminant from the feathers and access to clean water is essential; otherwise the plumage becomes soiled with fecal material, resulting in a loss of water-repellency once more. Many of the failures of rehabilitating oiled seabirds have apparently been due to the unsuitable conditions in which the birds were maintained after they had been cleaned. These cleaning and rehabilitation methods were incorporated in a booklet (RUROS 1972b) which had wide circulation. A number of ama-

teur naturalists subsequently used these methods successfully without further instruction or advice.

## Operational Practice

Much of the basic screening of cleaning agents was carried out in standardized tests on individual feathers and what at the time appeared to be the most satisfactory methods were used on the small number of oiled birds that were received by the Unit. Serious attempts to clean and rehabilitate oiled birds were deferred until a satisfactory treatment had been devised and this was then used operationally in a number of oil pollution incidents (RUROS 1973; Clark and Croxall 1972).

In one such incident when oiled guillemots came ashore on the south Devon coast in January 1972 some were treated locally, but others, a group of 14 birds, were brought to Newcastle for treatment. The birds were kept warm and fed at intervals of 4–5 h. The day after they had been collected from the beach they were taken by road to Newcastle, a distance of about 700 km, rested for 2 d, and only then cleaned. One bird died a week later, but the remainder were transported back to Devon 27 d after they had been captured. Nine were released immediately and two others at a later date. The two remaining birds were retained at an RSPCA center in the southwest where they died a few weeks later.

In a second incident in the Firth of Forth in February 1972, a large number of scaup (*Aythya marila*) came ashore contaminated with a light fuel oil. There were inadequate facilities for keeping the birds during the week in which they were coming ashore and being cleaned, and 50 birds of the 247 treated died. It was then possible to transfer the survivors to the Edinburgh zoo where better facilities were available. Eighty-two more scaup, which were in poor condition, died there, but the remainder rapidly improved and the intention of ringing the survivors was frustrated because most of them flew off in the course of the next week.

These experiences emphasized the futility of attempting to rehabilitate oiled seabirds unless appropriate facilities can be provided. These include shelter, warmth, and food for the birds immediately after capture, an abundant supply of hot water for cleaning the birds, and large pens with clean bathing water during recuperation. They cannot usually be provided without advance preparation, although as numerous examples since the introduction of these methods of treatment have shown, it is quite possible for the interested amateur to treat successfully small numbers of oiled birds.

Even a relatively small quantity of oil, however, may well result in tens, hundreds, or even thousands of oiled birds coming ashore. Many may be beyond recovery, but numbers of this magnitude cannot be handled in small or improvised centers. If realistic rescue operations are to be mounted in these circumstances, they require extensive facilities, labor, and organization. Fortunately, as our experience has shown, oiled birds can be transported long distances so that a permanent treatment center, strategically placed, with appropriate facilities, good communications, and conveniently located for the volunteer help that would be needed in a crisis, can serve a considerable area of coast.

Following the oil spillage in the Firth of Forth in February 1972 there was considerable interest among Scottish animal welfare organizations to establish a permanent center to cover the firths of Tay and Forth where numerous sea ducks winter. The development of the North Sea oil field was seen as placing the east coast of Scotland under greater threat of oil pollution than before and it was envisaged that a second center serving the Moray Firth might be needed at a later stage. But with these two centers it was likely that the high-risk areas of the mainland Scottish coast would be adequately covered. Two similar centers in southwest and southeast England would serve the high-risk parts of the English coastline. By basing these centers on existing nature reserves or other facilities that already had a full-time warden, the costs of establishing and running them would have been relatively small.

In the event, some difficulty was experienced in negotiations for sites in Scotland and, in the absence of further oil spills at that time, interest in the proposal evaporated. Subsequently one such center was established by the RSPCA in the southwest of England near Taunton, but this has not so far been very active.

## Value of Rehabilitating Oiled Seabirds

Providing the birds are not too emaciated or ill when they are rescued from the sea, or more commonly the beach, and providing appropriate facilities exist for cleaning and rehabilitating them, most will recover and can be returned to sea in a healthy condition within 2 or 3 wk. Unfortunately we know very little about their subsequent fate. Because of the general policy in Britain against ringing "sick" birds, few cleaned birds have been ringed before release. However, all birds treated by the Research Unit were ringed and although the numbers of these have been too small to provide a reliable view of the success of cleaning, the returns have been of some interest.

Of 88 oiled guillemots cleaned and released in the 3 yr 1972–74, 7 were recovered dead within a week or two of release and 1 bird was found alive but in poor condition and probably did not survive. Rehabilitation was clearly unsuccessful in these cases. On the other hand, one bird certainly traveled 800 km to northwest France after release, two others were identified alive some 4 mo after release, and one more appears to have integrated with a local breeding colony. One colour-ringed kittiwake (*Rissa tridactyla*) released by the Unit certainly joined a nearby breeding colony. Although only 21 of the 114 scaup that escaped from Edinburgh zoo after cleaning were ringed, no dead scaup, ringed or unringed, were reported in the area during the weeks following their escape. In view of the close watch that has been kept on British mainland coasts for dead and stranded birds and

the reliable return of rings, there is a reasonable chance that many of the birds that are not accounted for have survived (RUROS 1973, 1974, 1975).

Whether or not this measure of success justifies cleaning and rehabilitating oiled seabirds and, in particular, justifies setting up permanent treatment centers, depends on the point of view that is taken.

From a conservation viewpoint, it has generally little value. There is no point, for example, in attempting to clean and rehabilitate gulls when their numbers are increasing rapidly in western Europe despite losses from oil pollution and when in several areas in Britain an attempt is being made to reduce gull populations because of the nuisance they constitute. Although the reported decline in numbers of sea duck migrating through Scandinavia was attributed to the effect of oil pollution (Clark 1969), most species have a high reproductive potential and recover rapidly from adult mortalities. Eider colonies on the island of Kökar were severely affected by oil when the tanker *Palva* ran aground in Finnish waters in May 1969: 25–33% of the breeding population was estimated to have been killed, yet in the following year the eider population on Kökar was exceptionally large (Leppäkoski 1973) and the oil spill had no long-term effect on them.

A number of boreal and subboreal species — auks and penguins in particular — are characterized by great longevity coupled with extremely low reproductive potential. Such birds are unable to restore quickly numbers reduced by adult mortality. Unhappily these species, through their gregarious and pelagic habits, are particularly exposed to heavy losses through oil pollution. They must claim high priority in any conservation measures that are attempted. Rescue and cleaning of oiled birds can make only a very small contribution to this. A large proportion of oiled birds fail to come ashore but die at sea, and of those reaching the shore many will be beyond recovery by the time they are found. Many factors influence this inescapable mortality — weather, distance from shore of the oil, accessibility of beaches, manpower — but in all but exceptionally favorable circumstances it is likely to represent the overwhelming majority of contaminated birds. Even if cleaning and rehabilitation are well organized and expertly carried out, it may be expected that only a trifling proportion of the oiled birds can be rescued and returned to the natural environment. Conservation clearly demands more direct measures of dispersing oil slicks before they reach sensitive areas, exploring the possibility of scaring birds from threatening oil as Koski and Richardson (1976)[2] have done, or, potentially most effective of all, devising methods of improving the extraordinary low reproductive success of these birds.

[2]This publication is available free from the Petroleum Association for Conservation of the Canadian Environment, 130 Albert St., Suite 400, Ottawa, Canada K1P 5G4.

It is conceivable that the rescue and cleaning of oiled birds would be justified for a species that was exceptionally rare and threatened with extinction, but in general it can make a negligible contribution to seabird conservation. Nevertheless, in a number of countries there is strong public pressure to mount rescue operations when seabirds are oiled. The motive for this may be humanitarian, even sentimental, and has little scientific basis, but it cannot be ignored because of this. At least if oiled birds are to be rescued and treated, we now know how it can be most effectively and economically done.

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*SESSION II. Summary and Overview*

# Physiological Stresses and Response in Chronically Oiled Organisms

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## Microbial Degradation of Oil

Session I shows that these processes apparently occur at low rates in anaerobic layers of sediments and generally in arctic environments, compared with surface sediments in high- and low-energy environments. Microbial degradation of paraffins occurs more rapidly than the degradation of aromatic hydrocarbons, and activated local microbial populations can result in significant losses of hydrocarbons (Vandermeulen and Penrose; Gordon et al.). $C_{17}$/pristane and $C_{18}$/phytane ratios may be useful indices for comparing degradative changes in different habitats (~8 mo, Cretney et al.). Hopefully, it will be possible for workers using gas–liquid chromatography (GLC) to make these calculations, so that comparisons of different environments can be made.

Sediment analyses and microbial data show that aromatic hydrocarbons are ultimately degraded, but what are the mechanisms involved in these relatively slow processes (Stewart and Marks)? The picture is surprisingly consistent regarding the general degradation pattern of aromatic hydrocarbons in sediments from naphthalene through alkyl phenanthrenes.

## Effects of Oiled Sediments on Field Populations

Recolonization and recruitment studies have demonstrated "real world" effects of petroleum exposure on organisms. Decapods, amphipods, isopods, bivalves, and polychaetes have been studied in this regard (Atlas et al.; Gordon et al.; and Gilfillan and Vandermeulen). Differences in experimental parameters, such as time scales, analytical chemical methods, suitable control groups (e.g. knowledge of the pre-spill situations), and various environmental factors make comparisons difficult.

Relations of size distributions and total population densities to contents of alkyl naphthalenes in sediment appear to be present (*Uca*). In other cases, rather high concentrations of hydrocarbons in sediments are associated with a large population of *Arenicola* (Gordon et al.). These findings lead to an interest in topics relating to routes of hydrocarbon transport to organisms, their magnitude and the retention times of the hydrocarbons.

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## Bioavailability of Sediment-Bound Hydrocarbons

Detritus feeders do not appear to show tissue magnification of hydrocarbons, even after long-term exposures to oiled sediments. A possible exception may be benzo[*a*]pyrene (Roesijadi et al.). Uptake of hydrocarbons from sediments has been demonstrated for polychaetes, bivalves, crabs, and fish; however, the tissue concentrations are generally well below those of the sediments. This circumstance has been demonstrated in field situations where inputs and outputs are not readily measured. When there is evidence for hydrocarbon renewal, the tissue concentrations are enhanced (Krebs and Burns). Only when the interstitial water, receiving inputs from sediment leaching (via whatever route), is not highly and rapidly diluted is uptake to be expected. Thus, the degree of organism contamination from hydrocarbons decreases as the extent of petroleum weathering (degradation) in sediments increases. Moreover, all chemical and biological factors that control the types and rates of degradation and release of aromatic hydrocarbons from the sediments influence the duration and composition of tissue contaminants. If this hypothesis (Anderson et al.) is correct, there are interesting implications regarding various efforts toward sediment recovery. Natural or human actions leading to aeration or removal of anaerobic layers contaminated with oil may speed recovery times.

## Tissue Retention of Hydrocarbons

Explanations seem to be available for the depuration rates of various organisms on the basis of the activities of degradative enzymes (aryl hydrocarbon hydroxylases, AHH) or relative partitioning of specific hydrocarbons between tissue lipids and the water phase. In most cases, organisms with relatively high AHH activities (fish and crustaceans) apparently dispose of hydrocarbons faster than those having lower AHH activities (bivalves). Notwithstanding, losses in hydrocarbons may reflect increases in the accumulation of metabolites. The only significant induction of AHH activity has been in pollutant exposed fish.

The degree of alkyl substitution and the number of aromatic rings influence the rates of hydrocarbon release from tissues, as previously shown in the literature. Two reports have suggested that sulfur-containing aromatic compounds (e.g. dimethylbenzothiophene) have relatively long retention times (in mussels and isopods). *Mya*, from sediments containing oil for several years, showed a rather long retention of a contaminant; however, the nature of the components was not elucidated. Before further addressing the question of food-web transfer from long-term contaminated organisms, more work needs to be conducted on the identification of accumulated hydrocarbons and metabolic products in invertebrates as well as consumer species.

## Means of Determining Effects on Biota

Decidedly less use is being made of 96-h LC50 values in the assessment of biological damage. The development and testing of more meaningful measurements of organismic and population responses have superceded the use of this simple index. More progress in ways of evaluating petroleum effects on organisms are clearly needed, but some approaches are proving to be quite informative. In many cases, realistic exposure systems and petroleum concentrations are being used. Problems of quantitation still exist, however, and difficulties in comparing results from different laboratories are apparent. The choice of analytical methods appears to be largely dependent on available facilities. Flowing-water and sediment-exposure systems have been devised and tested. The combination of field and laboratory work is most helpful in providing balanced views on petroleum pollution. Chronically contaminated areas are very valuable test sites, and should be utilized.

While the genetic aspects of populations may prove to be valuable means of evaluating effect, other ecological data on "health" of organisms should be related to the differences observed. Certainly a well-rounded perspective of biological effects would have been obtained if the genetic data compiled from snails were available for *Uca* to complement the population information and the hydrocarbon chemistry provided. Studies with flounder exposed to oiled sediment demonstrated that light and electron microscopies are useful tools in revealing petroleum-induced morphological damage and alterations in parasitic infections. Certainly, growth and reproduction of marine species are significant parameters to measure to determine pollutant effects. Work with different life stages often provides divergent findings which makes it difficult to extrapolate findings from one species to another. Data on tissue accumulations and metabolic capabilities of organisms (to include the evaluation of degradative enzymes and metabolites), coupled with studies of life stages, are important in the whole picture of petroleum pollution in aquatic systems.

## Recommendations

I. Strive for best possible analytical data in attempting to relate petroleum exposure to tissue accumulations and biological alterations. Hopefully, future work will include the chemical analysis of products of enzymatic degradation in tissues and the biological effects of petroleum weathered through chemical and microbial oxidations.

II. In studies of petroleum contamination via sediments, the nature of uptake processes through interstitial water are important. It is most practical to couple studies of chemical alterations of sediments with organismic, population, or community effects. Various experimental approaches can be tested during these long-term exposures to evaluate the sensitivity of a given parameter. Exposures could be either in the field or laboratory, but realistic levels should be used and water exposures should be flow-through systems.

III. In all studies, the "big picture" should be kept in mind, such that data will generate information on rates of habitat recovery and the levels of contamination that still allow "normal" community survival.

**Symposium on Recovery Potential of Oiled Marine Northern Environments**

*SESSION III*

## Long-Term Effects of Oil Spills on Marine Intertidal Communities

CHAIRMEN: K. H. MANN AND R. B. CLARK

# Recolonization of Rocky Shores in Cornwall After Use of Toxic Dispersants to Clean Up the *Torrey Canyon* Spill[1]

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SOUTHWARD, A. J., AND E. C. SOUTHWARD. 1978. Recolonization of rocky shores in Cornwall after use of toxic dispersants to clean up the *Torrey Canyon* spill. J. Fish. Res. Board Can. 35: 682–706.

Fourteen thousand tons of Kuwait crude oil, reduced from 18 000 tons by weathering at sea, was stranded along 150 km of the coast of West Cornwall, England, in March 1967. The oil was treated with 10 000 tons of toxic dispersants during cleaning operations. By itself the oil was not very toxic, although it killed some limpets and barnacles, and most of the mortalities that followed cleaning were due to the dispersants. There was a graded effect. Most animals and some algae were killed on the shores treated heavily with dispersants, while a few animals and most algae survived in places less heavily treated. However, long stretches of coast were contaminated to some extent by drifting of patches of oil and dispersants along the shore and by indiscriminate dispersant use in remote coves. The general sequence of recolonization was similar to that which has been found after small-scale experiments, where the rocks were scraped clean, or where limpets were removed, but took longer to complete. There was first a rapid "greening" by the alga *Enteromorpha*; then a heavy settlement and growth of perennial brown algae (*Fucus* species), leading to loss of surviving barnacles. A settlement of limpets and other grazing animals followed, with eventual removal or loss of the brown algae. The final phases were a reduction in the limpet population and a resettlement of barnacles. Lightly oiled, wave-beaten rocks that received light dispersant treatment showed the most complete return to normal, taking about 5–8 yr; heavily oiled places that received repeated application of dispersants have taken 9–10 yr and may not be completely normal yet. Most common species returned within 10 yr, but one rare hermit crab is still missing from places directly treated with dispersants. The early recolonization by algae resulted in a raising of the upper limit of *Laminaria digitata* and *Himanthalia elongata* by as much as 2 m in wave-beaten places, demonstrating that grazing pressure by limpets must be one of the factors controlling the zonation of these plants. Later, other species of plants and animals were found higher up the shore than usual, under the shade and shelter provided by the dense canopy of *Fucus*. Fluctuations in the populations of algae and herbivorous animals during the course of the recolonization illustrate the importance of biological interactions in controlling the structure of intertidal communities. Pollution disturbance affects the herbivores more than plants, hence the point of stability of the community is shifted towards the sheltered shore condition of low species richness and greater biomass.

*Key words:* petroleum, dispersant, rocky shore, *Torrey Canyon*, recolonization, coastal ecology, pollution

SOUTHWARD, A. J., AND E. C. SOUTHWARD. 1978. Recolonization of rocky shores in Cornwall after use of toxic dispersants to clean up the *Torrey Canyon* spill. J. Fish. Res. Board Can. 35: 682–706.

Quatorze mille tonnes de pétrole brut de Koweït, provenant de 18 000 tonnes réduites par altération à l'air en mer, se sont échouées sur 150 km de côtes dans la partie ouest de Cornouailles, Angleterre, en mars 1967. Au cours des opérations de nettoyage, le pétrole fut traité à 10 000 tonnes de dispersants toxiques. Le pétrole n'était pas par lui-même très toxique, bien qu'il causa la mort de quelques patelles et balanes. Les dispersants furent responsables de la plupart des mortalités qui suivirent le nettoyage. Il y eut effet progressif. Sur les rivages lourdement traités aux dispersants, la plupart des animaux et quelques algues furent tués, alors que, aux endroits moins lourdement traités, quelques animaux et la plupart des algues survécurent. Cependant, de longs segments de la côte furent plus ou moins contaminés par la dérive de pétrole et de dispersants le long du

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rivage et par l'emploi à tort et à travers de dispersants dans des anses éloignées. La recolonisation se fit en général suivant le même ordre que celui qui avait été observé dans des expériences à petite échelle dans lesquelles des rochers avaient été nettoyés et les patelles enlevées. Elle se fit toutefois plus lentement. Il y eut d'abord un « verdissement » rapide par l'algue *Enteromorpha*; ensuite une abondante colonisation et rapide croissance d'algues brunes vivaces (espèces de *Fucus*), entraînant une perte de balanes qui avaient survécu. Vint ensuite une colonisation de patelles et autres brouteurs, causant éventuellement l'enlèvement ou la perte des algues brunes. Les phases finales comportèrent une réduction de la population de patelles et une nouvelle colonisation de balanes. Les rochers battus par les vagues et légèrement touchés par le pétrole, qui ne reçurent qu'un léger traitement aux dispersants, sont ceux qui retournèrent le plus près de la normale, après environ 5 à 8 ans; les endroits fortement touchés par le pétrole et qui reçurent une application répétée de dispersants prirent 9 à 10 ans à se rétablir et ne sont peut-être pas encore revenus à la normale. La plupart des espèces communes étaient revenues en dedans de 10 ans, mais un Bernard l'Ermite rare manque encore aux endroits directement traité aux dispersants. Comme conséquence de la recolonisation par les algues, la limite supérieure de *Laminaria digitata* et d'*Himanthalia elongata* s'est élevée d'une distance allant jusqu'à 2 m aux endroits battus par les vagues, ce qui démontre que le broutage par les patelles doit être un des facteurs contrôlant la répartition en zones de ces plantes. Plus tard, on a trouvé des espèces de plantes et d'animaux à un niveau plus élevé que d'habitude sur le rivage, profitant de l'ombre et de l'abri fournis par de denses tapis de *Fucus*. Les fluctuations que subissent les populations d'algues et d'animaux herbivores au cours de la recolonisation démontrent bien l'importance des interactions biologiques dans le contrôle de la structure des communautés intertidales. La perturbation causée par la pollution affecte les herbivores plus que les plantes. Il en découle que le point d'équilibre de la communauté se déplace vers une condition abritée des rivages, à faible diversité d'espèces et abondante biomasse.

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THE staff of the Marine Biological Association (Smith 1968) have described the immediate consequences of the release of 119 000 tons of Kuwait crude oil from the tanker *Torrey Canyon* which grounded on the Seven Stones reef off the western tip of Cornwall in 1967; the report was compiled and published within a year of the disaster, and little could be said about recolonization of denuded shores or long-term changes in the ecosystem. Later accounts of the situation in Brittany (Bone and Holme 1968; Stebbings 1970) and at Porthleven (Bryan 1969) described events up to 18 mo after the spill, but were also unable to deal with long-term changes. More recent information has been reported at oil pollution symposia and in general reviews (Nelson-Smith 1968, 1972; Spooner 1970, 1971; Crapp 1971d). To balance gloomy forecasts of eco-disaster made in 1967, attention was drawn to the rapidity of the start of recolonization. Unfortunately, the use of the term "recovery" seems to have encouraged later writers to believe that all was now normal on Cornish shores: e.g. "recolonisation by animals from unpolluted cases . . . began almost at once, slowly at first but then more rapidly. By the time of the Santa Barbara oil spill (i.e. February 1969), recovery of most of the polluted areas seemed essentially complete" (Steinhart and Steinhart 1972); "considering the remarkably short period used up in recovery . . . of the natural communities" (Mackin 1973); "the damage was almost completely restored in one year, and returned to normal after a few more" (Wardley Smith 1976), "with exception ("Tampico Maru" spill) intertidal communities appear to have been affected least and to have recovered the fastest . . . usually within two years" (Hyland and Schneider 1976). These authors overlooked a serious contribution by Cowell et al. (1972) who "expected recovery to be very protracted," as indeed it has been in Cornwall.

Now, after 10 yr the affected shores in West Cornwall have achieved some degree of normality, which is what we understand by the word "recovery." Hence it is at last possible to give an account of the recolonization of the intertidal zone. In this contribution we describe some of the broad-scale changes observed on rocky shores and relate them to more detailed studies of fluctuations in population abundance of barnacles, limpets, and other common organisms at selected stations. Some additional data are given on the fate of the oil, and of events in other habitats, derived from information kindly supplied by colleagues.

Taxonomy is based on the Plymouth Marine Fauna (Marine Biological Association 1957) and on Parke and Dixon (1976); authors' names and references to species are quoted only where a different usage is employed. It has been impossible to name the encrusting coralline algae; they are referred to here collectively as "lithothamnia," as in general works on intertidal ecology.

## The Oil and the Dispersants

The amount of oil released by the *Torrey Canyon* spill has not been exceeded since, though it was almost equalled by the *Urquiola* disaster at La Coruna in 1976 (Quiroga 1976). About half of the cargo did not reach the shore, and was sunk at sea after weathering or was dispersed by natural agencies (Table 1, Fig. 1), but

TABLE 1. Chronology of the *Torrey Canyon* oil spill.

| | |
|---|---|
| Mar. 18, 1967 | *Torrey Canyon*, carrying 119 000 tons Kuwait crude oil (S.G. 0.869) stranded on Seven Stones reef. |
| Mar. 20, 1967 | 31 000 tons of oil released up to this date, some treated at sea with dispersants (68 000 $dm^3$). |
| Mar. 25, 1967 | First oil came ashore in Cornwall. |
| Mar. 26, 1967 | Cumulative total of 49 000 tons of oil released from wreck; the last 18 000 tons went towards Cornwall and lost 4000 tons by evaporation of the lighter fractions. |
| Mar. 26–28, 1967 | 40 000–50 000 tons more oil released as ship breaks up; most of it never reached the shore and was ultimately sunk or weathered in the Bay of Biscay. |
| Mar. 28, 1967 | By this date an estimated 14 000 tons of oil had come ashore in West Cornwall; 10 000 tons of dispersants were used to clean it up. |
| Apr. 6–11, 1967 | The oil released up to March 20 now came ashore in the Channel Islands and North Brittany, an estimated 20 000 tons after evaporation. |
| May 19–20, 1967 | A small quantity of the oil (300 tons) released from the wreck in the period March 26–28 now came ashore in West Brittany; the rest was sunk by treatment with stearated chalk, or was lost in the North Atlantic ocean. |

some 40 000 tons is still believed to have come ashore in Cornwall, Brittany, and the Channel Islands (Smith 1968; Cabioch 1971). What distinguishes the affair from many other oil spills is not the quantity of oil that came ashore, but the quantity of dispersants (referred to as "detergents" at the time) used to remove oil from the beaches and rocky shores. There were undoubted instances of organisms being killed or damaged by the oil alone (O'Sullivan and Richardson 1967), but the relatively low toxicity of the oil is believed to have been further reduced by evaporation of the lighter and aromatic constituents during the interval of 1 wk or more between release and stranding (Smith 1968; Corner et al. 1968; but cf. Spooner 1971). Thus, although a little oil has persisted for some years, most of the effects observed on Cornish shores are due to short massive kills by dispersants applied in the spring months of 1967.

## PERSISTENCE OF THE OIL

A small quantity of the *Torrey Canyon* oil remained on the Cornish coast after dispersant application ceased, and a larger quantity was left on the shore in Guernsey and Brittany when cleaning operations ended there.

On dispersant-treated shores a little oil often remained on the rocks for some months, as for example at Porthleven and Trevone, but this weathered as fast as the apparently untreated oil at Godrevy Point and little was left after 1 yr. In contrast, oil driven into beach sands by dispersant treatment, buried by bulldozing, or covered by natural sand movements, persisted for several years. In 1968, oil was surfacing at Whitesands, Sennen, between mean tide level (MTL) and high water neap (MHWN) causing iridescent streaks; and in the harbor at Porthleven, greater quantities of oil were found 3–5 cm deep, easily revealed by turning over large stones. Both of these superficial oil seepages disappeared by 1976. In a small area at Watergate Bay, on the north coast of Cornwall (between station 9 and Trevone on map, Fig. 2), weathered oil was uncovered by natural beach movements in 1970, having undergone very little biological degradation (Spooner 1971); the same oil was exposed again in 1971 and 1972 (Spooner personal communication). A smaller trace of oil was found off St. Ives in 1971, on dead shells of *Ensis* buried 15 cm down in the sandy bottom at 10 m depth of water (Forster personal communication). In view of the resistance of crude oil to degradation once it has sunk below the fully oxygenated layer in the sand (Johnstone 1970), it is likely that traces still remain today on the Cornish coast, buried in the sands and the immediate sublittoral.

Untreated oil survived for over a year on north Brittany shores, on high water rocks, sands and salt marshes, but considerable weathering occurred in this time (Bone and Holme 1968; Stebbings 1970). Similar patches of oil survived several years on some partly sheltered high water rocks on the west coast of Guernsey, but the amount still present in 1971, the last year photographed, was very small, the bulk of it having weathered away (Forster and Swinfen personal communication).

In the estuary at Hayle (between 11 and 12 on map, Fig. 2), which was not treated with dispersants (Smith 1968), a thin coating of oil on a sea wall had disappeared by 1969; in contrast, in a very sheltered region at the head of the creek, at extreme high water spring tide level, there were still patches of well-weathered asphalt-like oil 1 cm thick in 1970 (Spooner personal communication).

All of these examples of persistent *Torrey Canyon* oil represent a very small fraction of the original, not at all comparable with the amounts that have persisted after other oil spills.

## THE DISPERSANTS AND THEIR EFFECTS

The technique for dispersing oil patches on the shore by treatment with high aromatic solvents containing a nonionic surfactant, followed by hosing down, was developed by physicists at the Warren Springs Laboratory, England (Wardley Smith 1962). Tests at the MAFF Fishery Laboratories showed that the mixture could be toxic to shellfish, but it was felt that rapid dilution in the sea after application would prevent any damage to demersal or pelagic fish. The dispersing agents were therefore approved for use except in the vicinity of shellfish

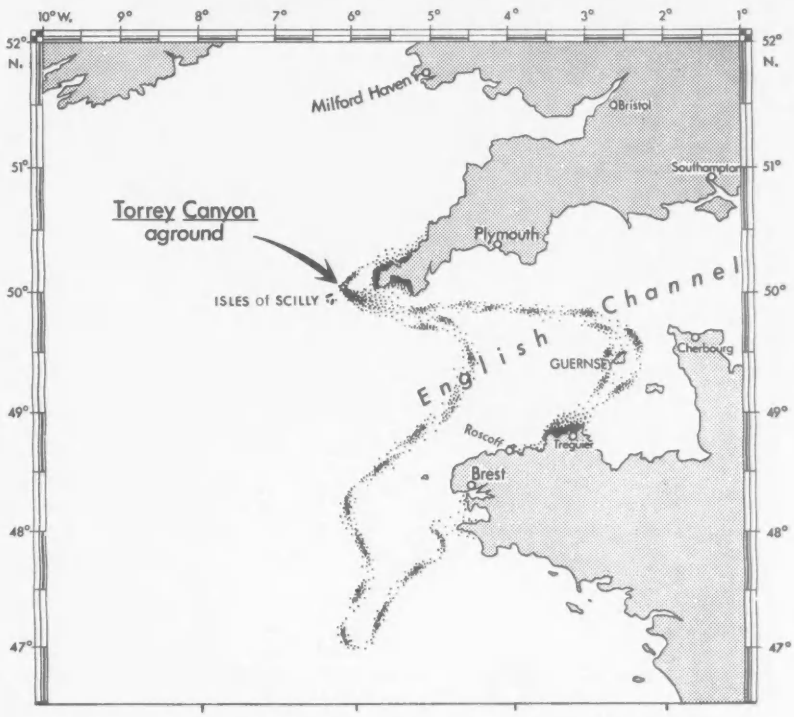



FIG. 1. Approximate track of the oil from the *Torrey Canyon*, and the places where it came ashore; see also Table 1. Reproduced by permission of the Marine Biological Association from Smith (1968).

beds, and their application became official policy. Unfortunately the results of the toxicity tests were distributed only in mimeographed reports, and not published until after the *Torrey Canyon* cleaning was over (Simpson 1968; Portmann and Connor 1968). In the emergency few people were informed of the toxicity of the dispersants to all forms of life, and indeed some of the spraying was carried out without enough regard for the safety of the operators themselves (cf. instructions in Warren Springs Laboratory, 1963 with those in Wardley Smith 1968). Scientific and public concern at the widespread use of dispersants was therefore directed at the surfactant component, initially based on published reports of experiments with freshwater life (e.g. Marchetti 1965). The extreme toxicity of the dispersants to marine life soon became apparent through field observations and hastily arranged laboratory tests. Today there is much published information on this point of which we were then ignorant (Boney 1968; Baker 1971; Crapp 1971b, d; Ottway 1971; Portmann 1972; Brown 1973; Beynon and Cowell 1974; van Gelder Ottway 1976). The most commonly used dispersant during the *Torrey Canyon* clean up contained 12% nonionic surfactant and 3% stabilizer in a high aromatic solvent ("kerosene extract"). The mixture was shown to have a 24-h $LC_{50}$ varying from 0.5 to 5 ppm on sublittoral organisms, and from 5 to 100 ppm on intertidal organisms. Over short periods the solvent was the most

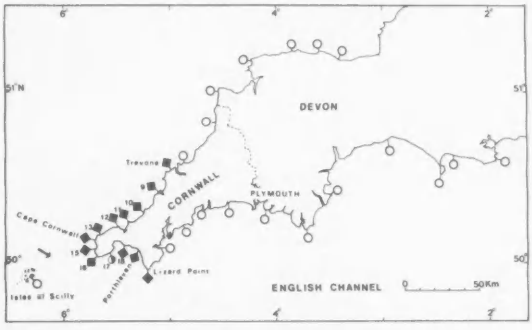



FIG. 2. Rocky shore stations investigated each year in southwest Britain since 1955. Black rectangles, much affected by *Torrey Canyon* operations; half white circle, slightly affected; white circles, free of *Torrey Canyon* effects. Numbers: 9, Newquay; 10, Chapel Porth; 11, Godrevy Point; 12, St. Ives: 13, Pendeen; 15, Sennen Cove; 16, Porthgwrra; 17, Lamorna Cove and Mousehole; 18, Marazion (Perranuthnoe is just to the east).

toxic component (Corner et al. 1968), but over long periods all components were very toxic (Wilson 1968). Most of the differences in sensitivity between species were fully supported by field observations, and the most sensitive common animals were the limpet *Patella vulgata* intertidally (5 ppm) and the razor shell *Ensis siliqua* sublittorally (0.5 ppm). The concentrations at which the dispersants were toxic to all life were very much lower than those required to emulsify the oil (1 part of dispersant to 2–4 parts of oil) and very much lower still than the ratio actually applied in the field (10 000 tons of dispersants to 14 000 tons of stranded oil).

The one redeeming feature of the dispersants, shown by laboratory testing, was the rapid loss of toxicity of the solvent fraction when exposed to the air, though the surfactant and stabilizer were more persistent (Corner et al. 1968; Wilson 1968). However, field studies depressingly confirmed the results of the laboratory tests, and showed that close to dispersant spraying practically all animal life was killed, while many algae, including members of the Chlorophyta, Rhodophyta, and Phaeophyta were killed or damaged. The herbivorous gastropods, the decapod crabs, and the echinoderms seemed to be the worst affected, and on places cleaned thoroughly hardly a living animal could be found. Thus on some rocky shores recolonization started from scratch; on others, treated less heavily, some fucoid algae, the more hardy animals such as barnacles, some sea anemones, the top-shell *Monodonta*, and occasionally a few limpets survived. There was thus a graded effect which would influence recovery, most severe on heavily treated shores close to tourist centers and especially around high water mark, and least severe on a few stretches of steep cliffs or along the outer edge of reef platforms at low tide level.

In theory, in view of the rapid decline in toxicity of the solvent component, recolonization of affected shores could have begun within a week or two of the last application of dispersants, and in fact settlement of the larvae of the barnacle *Balanus balanoides* was seen at some places early in May. However, rocky shores with easy access by road were treated several times, a few of them for up to 3 mo after the first oil came ashore. The characteristic aromatic smell of the dispersant could be detected at Trevone and Sennen Cove, for example, in June. In such places there was the danger that the repeated applications would not only bring the total kill to 100% (cf. Crapp 1971b, c) of all organisms, but would lead to accumulation of the persistent components such as the surfactants and the higher boiling point aromatics in the sandy beaches (cf. Blumer et al. 1970) and in sand and gravel under boulders or in crevices on rocky shores. This aspect has to be borne in mind when considering the slower course of recolonization on some sections of the shores.

A final problem caused by the widespread use of dispersants is that it has never been fully possible to separate the effects of oil alone from the effects of dispersants. Although many parts of the West Cornish coast are either inaccessible or approached by roads unsuitable for heavy transport, dispersants were often carried by helicopter, and the opened drums simply rolled over the cliff (Brown 1972; Frost 1974). Discrete patches of oil–dispersants drifted alongshore with the tides and currents (Smith 1968) ensuring that intermediate stretches were also affected. At one time we thought that the short length of coast from Lamorna Cove to Mousehole would form a satisfactory control, but even here some oil came ashore late in the spill and was treated with dispersants (Bellamy et al. 1967). It is thus incorrect to refer in general to "recolonisation from unpolluted oases" when continuous lengths of coast were affected to some degree, and when many of the returning organisms had to travel considerable distances.

Recolonization of a shore can occur in four ways: (a) by migration of adults of mobile species from unaffected areas (cf. Dauer and Simon 1976); (b) by direct settlement of planktonic spores or larvae liberated by breeding organisms in unaffected areas; (c) migration of juvenile stages of species with direct development (i.e. without planktonic larvae); (d) rafting in of adults or their egg masses attached to floating seaweed or debris. The first process, as we have mentioned, was hampered by the lengths of coastline affected, as to some extent was the third, leaving the settlement of planktonic larvae and spores as the most likely initial stage of recolonization, with rafting as the next possibility. For planktonic stages much depends on life history, breeding season, and time elapsed between liberation and settlement. At temperatures experienced inshore in West Cornwall (annual range 8–16°C) limpet larvae take about 10 d from spawning to reach settlement stage, and barnacle larvae take about 20 d from hatching to settlement, hence possible travel distances of 30–50 km might be anticipated (cf. Crisp 1958). Distances of this order are within reach of the completely unharmed shores outside the damaged area of West Cornwall (Isles of Scilly to the west; beyond Trevone to the north; round the Lizard peninsula to the east). For species without planktonic larvae such distances represent formidable barriers, and we have to look closer, for possible spread from places treated less heavily with dispersants or from islets and reefs off the coast outside the main drift of dispersants.

## The General Course of Recolonization on Rocky Shores

Figure 2 shows the stations in southwest Britain that have been visited annually since 1955 to follow natural changes in selected intertidal animals, chiefly the barnacles (e.g. Southward 1967); records for some of the stations go back to the 1930s (Fischer-Piette 1936). Not one of the stations in the *Torrey Canyon* area escaped some damage due to oil or dispersants. We have selected certain of them to follow details of recoloniz-

TABLE 2. The time course of recolonization of rocky shores in Cornwall, expressed in years from the date of the *Torrey Canyon* disaster, March 1967

| | Lizard Pt. exposed (Vellan Drang) | Lizard Pt. sheltered (Polpeor Cove) | Porthleven, west of harbor | Maen Du Pt., Perranuthnoe | Sennen Cove exposed (300 m east of pier) | Sennen Cove (near Pier) | Cape Cornwall (Porthledden side) | Trevone, exposed ("sewer rocks") | Trevone, sheltered (MTL reefs) |
|---|---|---|---|---|---|---|---|---|---|
| Relative exposure to wave action | +++ | ++ | ++ | ++ | +++ | ++ | +++ | +++ | + |
| Amount of oil stranded | + | ++ | +++ | + | ++ | +++ | ++ | ++ | +++ |
| Dispersant treatment | (+) | +++ | +++ | (++) | ++ | +++ | ++ | ++ | +++ |
| *Enteromorpha* maximum[a] | 1 | 1 | 1 | 1 | 0–1 | 1 | 1 | 0–1 | 1 |
| Maximum *Fucus* cover | 2–3 | 1–3 | 1–3 | 2–3 | 1–3 | 1–3 | 1–2 | 2 | 1–3 |
| Minimum of barnacles | 2 | 2 | 3 | 4 | 3 | 3 | 3 | 2 | 2–6 |
| Maximum numbers of *Patella* | —[b] | 6 | 5 | 5 | —[b] | 3 | 3 | 3 | 5 |
| *Fucus vesiculosus* starts to decline | 4 | 4 | 4 | 4 | 4 | 5 | 3 | 3 | 4 |
| *Fucus vesiculosus* all gone | 5 | 6–7 | 6–7 | 6 | 5 | 6 | 5 | 5 | 8 |
| Increase in barnacles | 4 | 6 | 6 | 5 | 4 | 6 | 4 | 3 | 7 |
| Numbers of *Patella* reduced | —[b] | 6 | 8 | 7 | 6–7 | 8 | 7 | —[b] | 6 |
| Normal richness of species regained | 5 | 9 | 10[c] | 8–10 | 9 | 9 | 8–9 | 5–6 | 9–10[c] |

[a]Only Trevone could be visited often enough from Plymouth to be sure of the extent of the 'greening' the 1st yr.
[b]No quantitative data.
[c]Full richness of species probably not yet regained on the area surveyed.
( ) Dispersant treatment comparatively lighter than at other places given same score.

ation, and the sequence of events is summarized in Table 2. Our data are partly in the form of field notes, including counts of the commoner animals on quadrats of $1 \times 1$ dm and $1 \times 1$ m, and partly in the form of color transparencies. It is impossible to overemphasize the importance of good color photographs, including close-ups, in following changes of this nature.

The first opportunists to return were the green algae *Enteromorpha* and *Ulva*, seen during June and becoming very obvious through the summer and autumn. This "green flush" is by now well known to follow severe oil pollution or dispersant application on temperate zone shores (Bellamy et al. 1967; Smith 1968; Nelson-Smith 1968, 1972; Crapp 1971b, d, f; Baker 1976a, b). It is principally a sign that the grazing herbivores, such as limpets, top-shells, and winkles, are absent or greatly reduced in number. This can happen naturally on a small scale due to storms, changes in sand levels, scouring by sand and gravel, or excessive freshwater run-off. It may also be caused by pollution other than oil (Smyth 1968), in which case we must distinguish it from "greening" due to eutrophication, for which *Ulva* rather than *Enteromorpha* is the best indicator (Burrows 1971). The continuance of "greening" is dependent not only on absence of herbivores but on the facility for rapid reproduction possessed by these relatively simple plants whose gametes and spores show peaks of abundance related to spring tides (Christie and Evans 1962; Rhyne 1973; see also Knight and Parke 1931). On some shores, e.g. Trevone and Sennen Cove, the green algae did not immediately recolonize the inner sheltered parts of the reefs, which showed maximum "greening" the next year. It is conceivable that this delay was due to persistent traces of dispersants adsorbed into gravel and sand under boulders or in silt in crevices in a concentration sufficient to damage the motile spores or gametes which resemble unicellular algae and may have a similar low resistance to toxins (cf. Smith 1968; Vandermeulen and Ahern 1976).

During the late summer and autumn of 1967 the large brown algae *F. vesiculosus* and *F. serratus* began to succeed the green algae. The first of these species exists in at least two forms or varieties in northwest Europe. In sheltered places the plants are large and carry air bladders (vesicles) along the fronds. In wave-beaten places, where limpets and barnacles are abundant, the plants are much smaller, without air bladders, and are usually referred to as f. *linearis* (=*evesiculosus*) (Lewis 1964; Boney 1966). From December 1967 through the spring of 1968 the outer parts of the reefs were browned by dense growths of f. *linearis*, while in less damaged sheltered shores f. *vesiculosus* also flourished (Fig. 4, 5, 6; Fig. 16, 17). This succession of the "green flush" by fucoids is also well established now for oil-polluted temperate shores after dispersant treatment (e.g. Crapp 1971b; Nelson-Smith 1972; Baker 1976b). However, places where greening was late or prolonged to the second spring usually failed to develop a growth of *F. vesiculosus* during the 1st yr. It is not certain if this was due to the absence of *Entero-*

*morpha* which provides protection from dessication at a critical stage in spore development after settlement (Knight and Parke 1950) or if residual dispersant toxicity might have remained long enough to inhibit settlement of spores during the summer fruiting season.

Under the dense mat of green and brown algae covering the affected shores in 1968 animal life was much reduced compared with that existing before the disaster. A few limpets had survived in places, and the cleared patches they maintained in the *Enteromorpha* by browsing allowed some barnacle settlement, but otherwise the barnacles that had survived the cleaning operations were gradually overgrown by the *Enteromorpha* and *Fucus* and eventually died. We do not know exactly why barnacles cannot live under a canopy of *Fucus*. It may be due to screening from the planktonic organisms on which the barnacles feed (Moore 1934), or physical effects such as abrasion of the young stages by the algal fronds when they are moved about by waves (Menge 1976). Another possibility is that the shelter and shade provided by the algae might allow the dog-whelk *Nucella* to feed on the barnacles for a longer period at low tide, and it is significant that barnacles can occur in quite high densities under *Ascophyllum* and *Fucus* in parts of estuaries where *Nucella* is absent or less common (cf. Moore 1934).

The few limpets that had survived on the cleansed shores grew much faster than normal on the abundance of algal food provided, up to 10 times faster than usual, and during 1968 they were joined by an equally fast growing number of juvenile *Patella* which had settled at low tide level or in wet places during the winter (Fischer-Piette 1941, 1948; Jones 1948; Lewis and Bowman 1975; Bowman and Lewis 1977) and were now moving up into the *Fucus* belt. The species of *Patella* found in Britain and NW Europe can feed on a much wider range of plants than is usual in other parts of the world or among species of the related genus *Acmaea* (Branch 1976). They normally browse on microscopic diatoms or other unicellular algae and larger encrusting species, rejecting only a few such as *Ralfsia* (Southward 1964), but when the opportunity offers they will readily feed on large and small plants of *Fucus*. Smaller limpets, below 20 mm length can be found feeding on the fronds and stipes of the *Fucus*, but all sizes will gnaw away at the holdfast and lower part of the stipe, where their tooth-marks can easily be seen. When large numbers of limpets are present the grazing on *Fucus* becomes severe, and the stipes are cut through or weakened to the point where they are torn away by the waves (Jones 1948; Southward 1956).

The first settlement of *Patella* on the *Torrey Canyon* affected shores was locally abundant (8–12/dm$^2$) but the overall numbers were low, and so was their size, hence grazing was insufficient to prevent a further heavy settlement of *Fucus* during the summer and autumn of 1968 (Fig. 11). It was at this stage, spring and summer of 1968, that we observed striking changes in zonation levels.

A second and larger settlement of *Patella* occurred during the winter of 1968–69, but the still increasing growth of the *Fucus* canopy prevented return of the barnacles, which reached their minimum in the years 1969 and 1970. The now rapid increase in limpet numbers and biomass (Fig. 12) restricted further settlement of *Fucus* in 1969 and later years, so that from 1970 to 1972 there was a rapidly ageing and thinning canopy of *Fucus* providing food for a quickly growing population of limpets underneath. From 1972 to 1974 most shores began to lose the *Fucus* dominance as the limpets removed or weakened the plants, and the last shores became "bare" in 1975.

In the absence of large algae the now enormous population of big limpets was reduced to feeding on microscopic and encrusting plants again, and there was not enough food to sustain them all. Many of them reacted by departing from their semisedentary ways (cf. Aitken 1962) and formed huge migrating "fronts" which worked their way across the reefs (Fig. 7) attacking the last remaining clumps of *F. vesiculosus*, and then gathering close to *F. serratus* persisting in pools and wet places (Fig. 14). Others, less adaptable, seem to have died or fallen easy prey to predators, and dead shells were washed up on some beaches during the winter of 1972–73. The typical limpet "fronts" were first seen at Porthleven in the winter of 1971–72, advancing westward, but similar "fronts" developed elsewhere in 1972 and 1973. The remnants of the "fronts" can still be recognized in 1977, gathered round pools containing *F. serratus*, having undergone a thinning out and stabilization since 1975 (Fig. 14). After the passage of these "fronts" across the survey areas, the population of *Patella* became reduced and the barnacle numbers steadily increased. From 1975 barnacles were dominant on most rocks, and algal growths seemed much reduced compared with before the disaster; in bright spring sunlight in 1976 and 1977 the shores appeared brilliantly white (Fig. 6, 13, 17).

Although the "greening" by *Enteromorpha* extended over such long stretches of coast, we were still surprised by the extent of the succeeding fucoid settlements observed in 1968 and 1969. At Porthleven, for example, it could clearly be seen from the cliff top that the *Fucus* dominance spread nearly 3 km west, almost to Trewavas Head, yet the main dispersant treatment in 1967 extended only 750 m west (see Bryan 1969). Comparatively late increases in fucoid cover occurred at other, lightly affected, stations where "greening" was not noticed in 1968, hence we have to raise the possibility that some of the increase in *Fucus* in Cornwall in 1968 and 1969 was a result of the heavy settlement the 1st yr bringing about a local increase in spore production. From the results of *Patella* clearance in the Isle of Man (Burrows and Lodge 1950; Southward 1956) it was suspected that the high density of *Fucus* on the experimental strips may have produced such a local abundance of spores that the surrounding rocks were colonized to a greater extent than would otherwise have been pos-

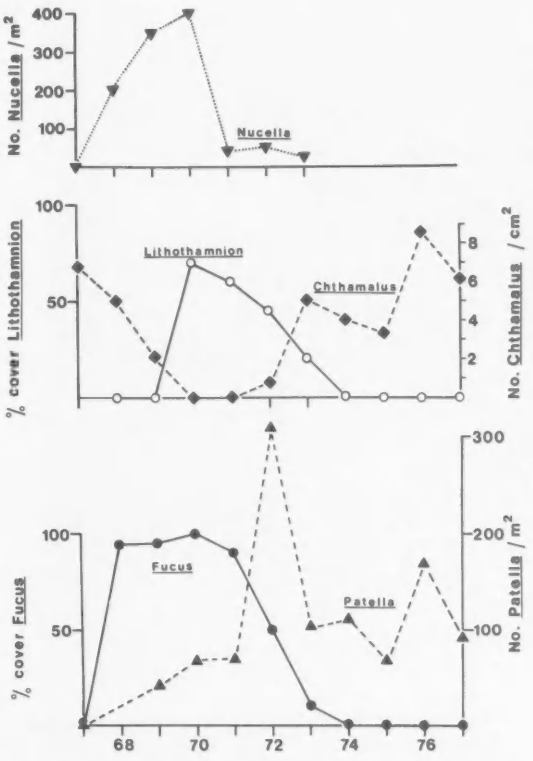



FIG. 3. Population fluctuations during recolonization on flat rocks at mean tide level at Porthleven from 1968 to 1977. Filled circles: percentage cover by *Fucus*. Filled triangles, no. of *Patella vulgata*/m²; open circles, percentage of rock covered by encrusting coralline algae ("lithothamnia"), filled rectangles, no. of barnacles (*Chthamalus*)/cm²; filled triangles and dotted line, no. of dog-whelks (*Nucella lapillus*) on a vertical surface near to the quadrats on which the other species were counted.

sible. If this happened in Cornwall, on the very much larger scale resulting from the disaster, it would help to explain the slower progress of the succession compared with the small experiments, and the longer period needed to regain stability.

### OTHER HABITATS

The foregoing description refers to the rocky midlittoral or eulittoral zone as defined by Lewis (1964) and Stephenson and Stephenson (1972). It should be noted that the lichens of the supralittoral fringe were damaged by dispersants and were slow to return to normal (Brown 1972, 1973, 1974). More localized damage resulted in maritime terrestrial communities where dispersants were spilled on the cliff tops during cleaning operations, and these have been equally slow to recover (Frost 1974).

The immediate sublittoral zone suffered drastic mortalities close to places where large quantities of dispersants were used (e.g. Porthleven, St. Ives), but recolonization by mobile fish and crustaceans was reported within a few months (Drew et al. 1967). The infauna of such sublittoral areas was slower to return (Forster personal communication), having to wait for favorable settlements of larvae, and the first repopulation by the heart-urchin *Echinocardium* was noticed 2 yr later. The razor-shell *Ensis* was slower to return, even though some migration by adults seems to have happened, and the last observations, in 1971, suggest the population was not as abundant as would normally be expected.

We have very little information on sandy beaches other than that reported in Smith (1968). There were no previous published data on the meiofauna of the coarse sand beaches affected by dispersants in West Cornwall, but mortalities are believed to have been heavy, and the beach structure was temporarily changed by dispersant treatment. Judging from laboratory experiments (Johnstone 1970; Bleakley and Boaden 1974) recolonization may have been slow.

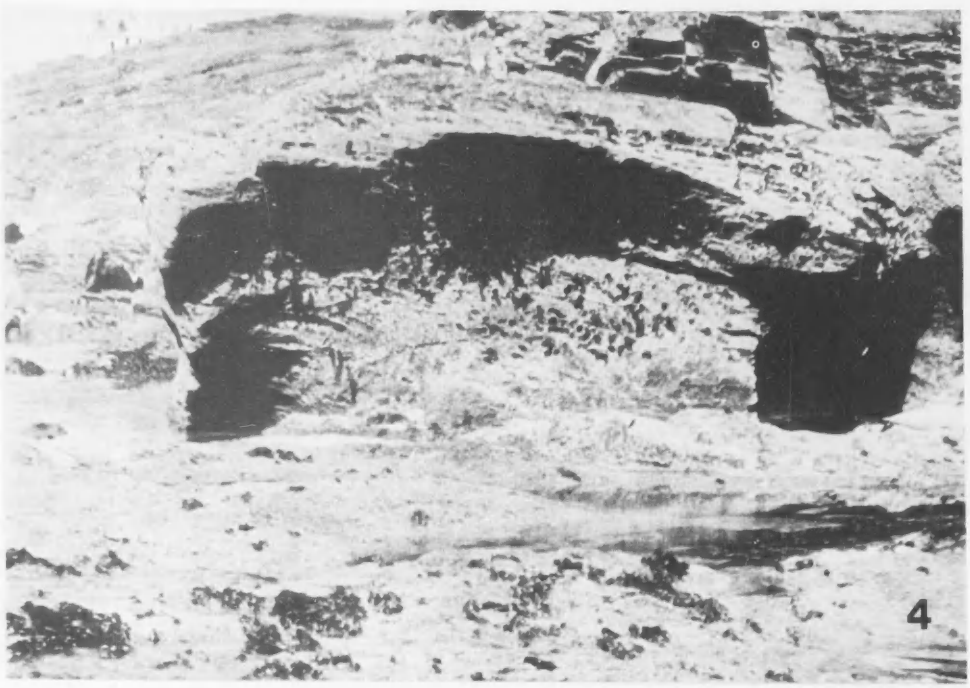
## Effect of Wave Action and Varied Dispersant Treatment on Recolonization

A range of rocky shores was investigated in detail, and the results selected here illustrate how local circumstances may influence the course of recolonization.

### PORTHLEVEN

The harbor region at Porthleven received very heavy dispersant treatment, and the rocks outside suffered from both direct treatment and drifting of partly diluted dispersant. The early course of recolonization by one species, the predatory gastropod *Nucella*, has been described by Bryan (1969), and we can now follow this through several more years. Some individuals at lower tide levels appear to have escaped the cleaning operations, and egg-cases were seen on the outer part of the reef at the end of April. By autumn 1967 a few adults with a shell showing "ledging," marks where growth was stopped for a while by dispersant effects, began to appear higher up the shore accompanied by larger numbers of juveniles which may have been survivors born before the disaster or may have hatched from the eggs seen in spring. These survivors and young specimens appear to have bred well, and by 1969 the population of *Nucella* was much larger than before the disaster (see Fig. 3); this may have been a result of lack of competition from other predators, or the shelter and shade provided by the growths of *Fucus*. In later years (Bryan personal communication, author's observations) the numbers of *Nucella* declined, perhaps as a result of the great reduction in the numbers of the barnacles on which they feed (Fig. 3).

As general recolonization of the inner parts of the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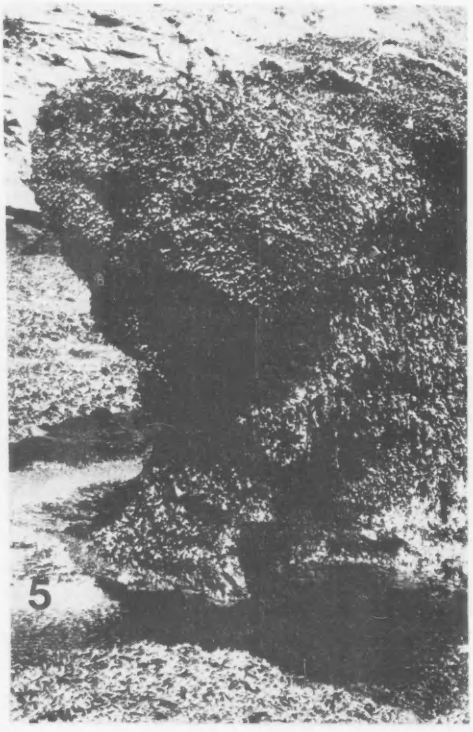
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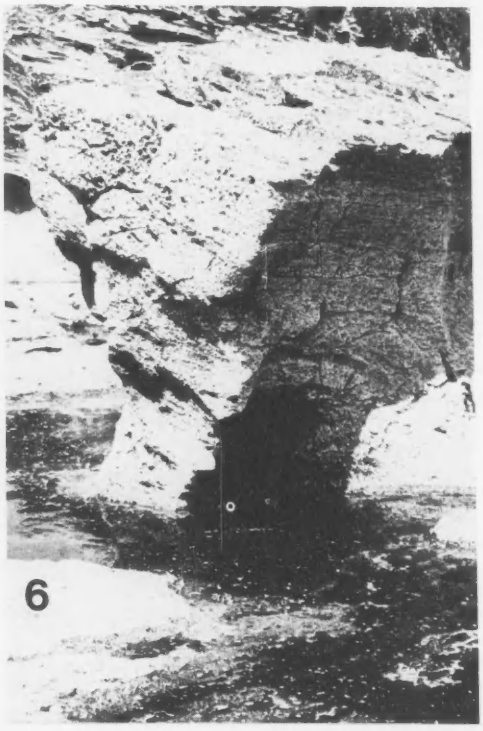
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FIG. 7. Porthleven, December 2, 1971, showing the "front" of *P. vulgata* "advancing" westwards towards the still dense growths of *Fucus* in the background.

reefs progressed, the area occupied by *Nucella* expanded. A single example was found near the harbor entrance (area d of Bryan 1969) early in 1970, and the species was found in somewhat greater number by the spring of 1973. This means that in the worst affected area, at least 34 mo were taken for recolonization, and by a species of considerable resistance to dispersants. In contrast, the decapod crabs which showed less resistance to dispersants, but which have planktonic larval stages and are more mobile in the adult stage, first began to return to the harbor area in November 1967, when they were found living under stones in the presence of persistent oil.

We have studied recolonization by several other species at Porthleven, mostly in the area of flat reefs at the most southerly point (area a of Bryan 1969). Quadrats were photographed and sampled annually each spring, and additional information on breeding of *Patella* was derived from autumn samples taken closer to the harbor. Details of the main sequence of changes at MTL are shown in Fig. 3 and some points illustrated in Fig. 4, 5, 6. It can be seen that 2 yr after the establishment of the *Fucus* cover, barnacles had disappeared from the horizontal rocks at MTL, and there was a corresponding drop in numbers of *Nucella* found on vertical faces nearby. It is noteworthy that the shells of *Chthamalus* have a high content of organic matter (Barnes et al. 1976) and do not persist long on the rocks after death; by 1969 all signs of the barnacle mortality clearly seen in 1967 had vanished. The population of *Fucus* began to decline first near the harbor (area b of Bryan 1969) in 1971, a region of irregular rocks where grazing by *Patella* was reinforced by development of a big population of edible winkles, *Littorina littorea*. The "front" of *Patella* migrating from this area (Fig. 7) passed across the quadrat region in the spring of 1972 and coincided with the first drastic decline of the *Fucus* there. A major resettlement of barnacles followed in the autumn, and by 1974 the rocks were virtually without algae. From 1975 to date the barnacle population has continued to increase while the limpets have declined, but the algal growths at MTL and MHWN are less than observed before the disaster (Fig. 6).

At Porthleven most plants below MLWN survived the more diluted dispersants received at this level, though the limpets were killed. Considerable algal growths, including some "greening," occurred in the first 2 yr, but by 1972 the mean low water spring tide

FIGURES 4, 5, and 6. Changes at Porthleven.

FIG. 4. May 8, 1967 after most of the oil had been removed by dispersants. The rocks look superficially normal, but in the absence of grazing molluscs, all killed, a film of diatoms and other small algae is beginning to darken the barnacle zone. Note the small tufts of *Fucus*, some of them now damaged but still illustrating the typical small-scale mosaic effect of the normal community on this shore. FIG. 5. May 12, 1968, a closer view of the southern corner of the rock outcrop seen in 4. Newly settled young plants of *Fucus vesiculosus* cover most of the rock, the remaining areas being bright green with *Enteromorpha* and *Ulva*. FIG. 6. May 18, 1977, the same viewpoint as 5, showing return to *Patella/Chthamalus* dominance. *Fucus* is now scarcer than before the disaster.

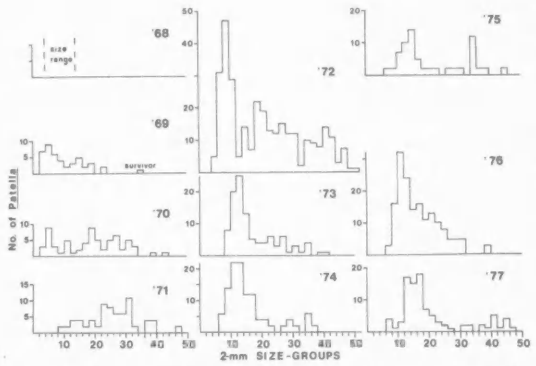



FIG. 8. Size-frequency histograms (in 2 mm shell length groups) for samples of *P. vulgata* from 1 × 1 m quadrats at mean tide level, Porthleven, from 1968 to 1977.

(LWS) region could be regarded as almost back to normal. In contrast, the HWS region took much longer to recover fully. Recolonization by *F. spiralis* did not reach a maximum until 1970, and these plants had not fully thinned out until 1975. The first littorinids to return were species with planktonic larvae, *L. neritoides* and small specimens of *L. littorea* (mistakenly identified as *L. saxatilis* in Smith 1968); these appeared late in 1967, but were more numerous by the spring of 1969. The smaller top-shell *Gibbula umbilicalis* also returned in large numbers (up to 24/m²) in 1969, and likewise has planktonic larvae. It was not until 1970 that species with direct development, other than *Nucella*, began to recolonize Porthleven, *L. littoralis* among the fucoids and a single genuine *L. rudis* Maton at the mean high water spring tide level (HWS). The first species is oviparous, and the eggs could have drifted in attached to seaweed; *L. rudis* is ovoviviparous (James 1968; Heller 1975), and the brood pouches contain developing embryos nearly all year round except in summer (Berry 1961; Bergerard 1971). The nearest population that is known to have survived is 4 km to the west, on a National Trust site that was lightly treated with dispersants. From 1968 to 1970 the HWS region carried a growth of *Enteromorpha* each spring, but with the return of all the littorinids and top-shells this greening disappeared. In 1971 *L. rudis* was present in slightly greater number, and by 1973 a local density of up to 6/dm² was found, and the harbor area had been recolonized. For some reason, the large top-shell *Monodonta* was slower to return than *G. umbilicalis*, though it also has planktonic larvae. A few escaped the cleaning operations and took up residence in crevices at HWN, but the flatter part of the reef was not recolonized until 1971, when small specimens were slightly more abundant on the rougher rocks to the east of the quadrat area. In 1972 the numbers increased to 6/m², and the species was abundant in 1973, when the numbers of *G. umbilicalis* began to fall.

Recolonization by the species of *Patella* was curiously local at Porthleven, and much influenced by microhabitat differences. On the MTL quadrats there was one possible survivor from before the disaster, but otherwise the population built up from new settlements each winter, starting in 1967–68 (see Fig. 8). The first new settlement reached 12–14 mm shell length by their second spring and up to 40 mm by the third spring, but really heavy settlement did not occur until the winter of 1970–71, by which time there was considerable bare rock below the lengthening and ageing *Fucus* plants. This last heavy settlement is seen as the modal class in spring 1972, accompanied by very large numbers of migrating adults. After this year the age-groups become increasingly confused by the slower rate of growth in the absence of algal cover, but the original settlements of 1967–68 and 1968–69 are still recognizable as small peaks on the size-frequency histograms at about 40 mm length. In 1977 these individuals would be 8 or 9 yr old, having grown very little during their last 5 yr. On many shores, as well as at Porthleven, this class forms the bulk of the remaining limpet "fronts" grouped near pools and wet places, and the shells show characteristic annual rings formed during the first few years when growth was rapid (cf. Fig. 14). During the years of *Fucus* cover all *Patella* shells showed signs of rapid growth in the protected and favorable habitat under the seaweed; they retained the protoconch, that relic of the larval twisted shell, for 2 yr or more, and although growth rings were present, the shell external surface was usually smooth and only slightly ribbed. Since the removal of the *Fucus* canopy the shells have become thicker and the erosion of the outer surface has brought the growth marks into greater prominence.

The most southerly distributed of the three species of limpet present on British shores, *P. depressa* Pennant (= *P. intermedia* Jeffreys), is a summer breeder (Orton and Southward 1961). New settlement was detected at Porthleven in the winter of 1967–68 but after 1969 this species was no longer found in the MTL quadrats dominated by *Fucus*. However, better settlements took place at MTL on some rather scoured rocks and concrete surfaces just outside the pier on the east side of Porthleven. The initial settlement of *P. vulgata* and *P. depressa* (240/m² by November 1968; 31% *P. depressa*) appears to have been great enough to prevent establishment of a general *Fucus* cover on these rocks. The mixed population received recruits in subsequent years up to 1972, when the sampling was discontinued; by this time the region was much nearer to normal than the main reefs to the west.

The third species of *Patella* in Britain, *P. aspera* Lamarck, is most characteristic of the lower half of the midlittoral zone on wave-beaten shores (cf. Lewis 1964). It was never abundant at the Porthleven stations except below MLWN, but a few specimens settled and grew at MTL on the flat reefs under the thinner cover of *Fucus* after 1970. These few large individuals were revealed on the quadrats when the *Fucus* disappeared but they themselves vanished after 1973, possibly mi-

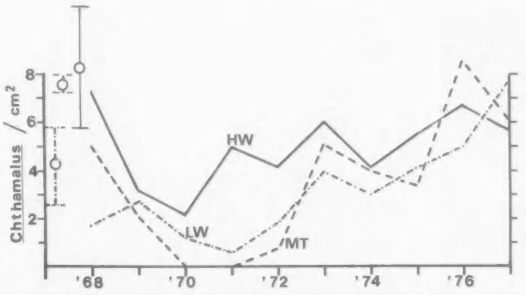



FIG. 9. Fluctuations in the numbers of *Chthamalus* at Porthleven from 1968 to 1977. Means and standard deviations are shown for counts in the years before the *Torrey Canyon* clean up. HW = mean high water neap tide level; MT = mid-tide level; LW = mean low water neap tide level.

grating into nearby pools where they would find congenial conditions. The counts of barnacles at Porthleven show some interesting differences between tide-levels (Fig. 9). At both HWN and MTL they declined to a minimum density in 1970. The HW region began to be recolonized sooner, though numbers are still below the range of abundance found before the disaster. The MTL region under its cover of *Fucus* was much slower to improve, but the numbers of *Chthamalus* reached their former level by 1976. At first the LWN region appeared to be unharmed, but the population declined later to a minimum in 1971. It has since built up to much greater densities than experienced before 1967, possibly as a result of the competing algae being reduced by the larger numbers of *Patella*.

## TREVONE

The extensive reefs at Trevone showed the maximum local differences in the time course of recolonization, in part related to intensity of dispersant treatment.

On the outer edge ("sewer rocks") not all the herbivores were killed, and just over 50% of the mussels and barnacles survived. However, there was an immediate "green flush" of *Enteromorpha* during the summer of 1967, though some settlements of *B. balanoides* and *Chthamalus* occurred. By 1968 the cover by *F. vesiculosus* f. *linearis* had increased and the barnacle population was reduced, but this change was quite short and the *Fucus* began to thin out in 1970, when barnacles and *Mytilus edulis* resettled in some number. Conditions were fairly normal by 1972–73.

In contrast, the partly sheltered MTL flat rocks half way out over the reefs have taken nearly twice as long to go through the cycle of recolonization. Some details are given in Fig. 10; it is noteworthy that the *Fucus* here consisted of very large plants of typical *F. vesiculosus*, with *F. serratus* among them (Fig. 11). The *Fucus* dominance lasted for 3 yr, and took a further 3 yr to disappear. As a consequence the barnacle zone on horizontal surfaces at MTL virtually vanished for 6 yr.

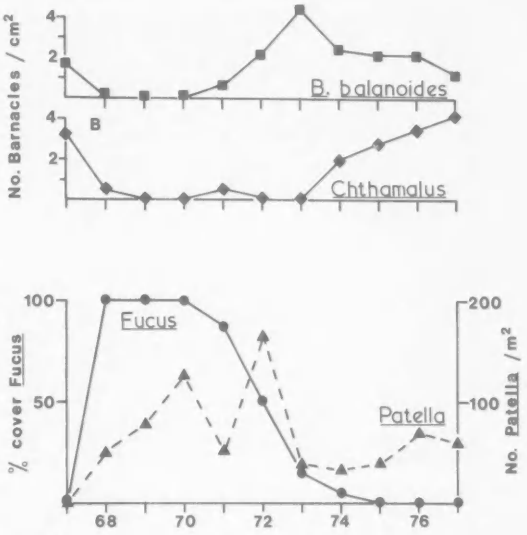
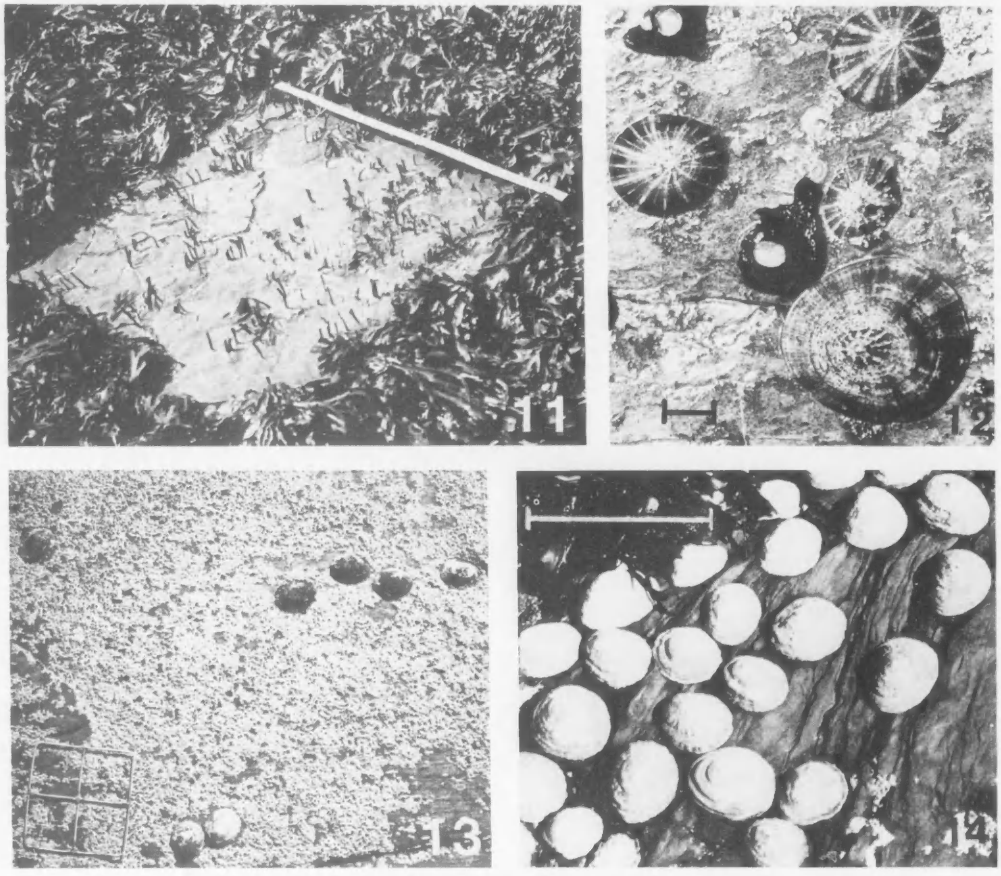



FIG. 10. Population fluctuations during recolonization of flat rocks at mean tide level at Trevone from 1968 to 1977. *Lower section*, circles indicate percentage cover by *Fucus*, triangles no. *P. vulgata*/m². *Upper section*, squares indicate no. *Balanus balanoides*/cm², diamonds no. *Chthamalus stellatus*/cm².

*Patella vulgata* never reached the same high numbers as at other stations, though its rate of growth appears to have been very fast (Fig. 12). In 1970 there had been a good settlement of *Spirorbis rupestris* under the *Fucus*, and *G. umbilicalis* had also returned. By the following year *G. umbilicalis* was present up to 40/m², and the *P. vulgata* had reached a maximum length of 50 mm, a remarkable size for 2½ yr growth. *Nucella lapillus* returned in 1969, but was more common in 1970; it may have been feeding on the numerous *Spirorbis* and the population of the low water barnacle *B. crenatus* which had now replaced the normal mixture of *Chthamalus* and *B. balanoides* previously present (Fig. 12). The first reduction of *Fucus*, in 1971, enabled *B. balanoides* to settle in some number, and it became the dominant barnacle for 3 yr (Fig. 10). The rocks were not really clear from *Fucus* until 1974, by which time the limpet population had fallen, and *Chthamalus* was then able to resettle (Fig. 13).

Of the limpets, only *P. vulgata* was present in the MTL quadrats during the first 5 yr at Trevone; *P. aspera* resettled in some numbers on the outer reefs in 1967–68 (up to 200/m², 9–23 mm length at MLWN in April 1968), but the southern species, *P. depressa*, was much slower to return than on the south coast of Cornwall, as for example at Porthleven and Perranuthnoe. The adult population in the southwest had been reduced by a succession of cold winters, and it is probable that the larval density in the plankton was lower along the north coast than on the warmer southern coast which carried more




FIGURES 11, 12, 13 AND 14. Fluctuations in algae and herbivores at Trevone.

FIG. 11. April 3, 1969. A metre quadrat has been cleared in the dense algal growths (20% *F. vesiculosus*, 80% *F. serratus*) just below mid-tide level. The rock under the algae is without barnacles, and the only fauna recorded was six small *P. vulgata*. Fig. 12. June 24, 1971. Close-up of part of a cleared quadrat at mid-tide level showing the rapidly grown *P. vulgata* now becoming abundant underneath the algal cover (90% *Fucus*). The rock surface carries many tubes of the worm *Spirorbis rupestris* and several *Balanus crenatus*, usually found only below low tide. Scale indicates 1 cm. Fig. 13. April 5, 1977, the same region photographed in 11 and 12, showing complete dominance of *P. vulgata* and mixed *Chthamalus/B. balanoides*. Wire frame is 10 × 10 cm. Fig. 14. April 24, 1975. An "aggregate" of old *P. vulgata* gathered at the edge of a shallow pool containing some plants of *F. serratus*. These limpets are survivors of the 1969 and 1970 settlements (as in 12), and now have rings on the shell showing how rapid was growth in the first 3 yr, and how little has taken place since. Scale indicates 10 cm.

breeding adults. A few did settle at Trevone in 1968 and later years, but we could find only 6/m² at the most in 1971. However, by 1975 the numbers of *P. depressa* had slowly built up, and the former comparatively large size for this species of up to 45 mm had been regained, though at 24/m² the abundance at the best station, an MLWN platform just inside the outer reefs, was less than in a previous period (Orton and Southward 1961). Further increases have followed, and this species is now present in small numbers over most of the rocks at Trevone.

One species that has not yet regained its former condition at Trevone is the long-lived brown alga *Ascophyllum nodosum*. In 1977 the surviving plants were only a tenth of the previous population existing at MLHWN below Atlantic Terrace. *Ascophyllum* has been shown to be sensitive to oil and dispersants and is slow to recover from cutting (Boney 1966, 1968; Keser et al. 1977; Steele 1977). It may never recover from the combination of *Torrey Canyon* effects and increasing recreational use of this particular stretch of shore (cf. Boalch and Jephson 1978).

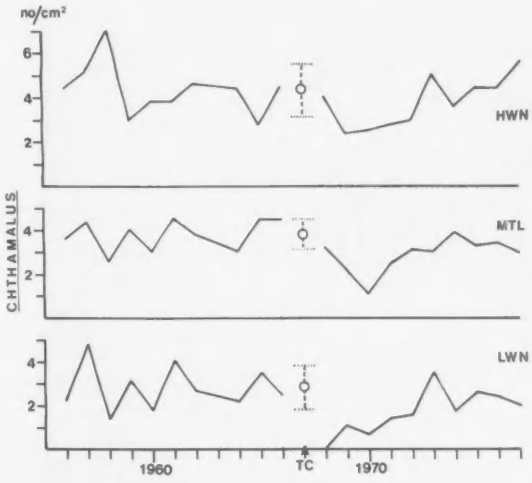



FIG. 15. Fluctuations in the numbers/cm² of *Chthamalus* at MHWN, MTL, and MLWN on the outer edge of the reef at Cape Cornwall from 1956 to 1977. Means and standard deviations are indicated for the 10 yr before the disaster. TC = no counts could be made in 1967, at the time of the spill.

### CAPE CORNWALL (AND SENNEN COVE)

A greater quantity of oil was stranded at Sennen Cove than at Cape Cornwall, but we have more biological information for the latter. The outer edge of the reef on the northwest side of Cape Cornwall has been surveyed each year since 1956. It is very exposed to wave action and normally carries a population of *P. aspera* as well as *P. vulgata*. In the MHWN to MTL region dense cover by *F. vesiculosus* f. *linearis* persisted only for a short time (see Table 2) after the disaster, but while present it was very thick. *Fucus spiralis* settled in the HWS region a year later than *F. vesiculosus* in 1968–69, but was never as dense. There was a heavy resettlement by *P. vulgata* and *P. aspera* in the first 3 yr, and this may have helped, together with the wave action, in shortening the period of *Fucus* dominance. As shown in Fig. 15, there was a less severe fall in the barnacle cover at this station than at Porthleven or Trevone. Changes were comparatively slight at the HWN level, where the spaced-out *Fucus* plants allowed some recruitment, and the numbers have been back at their normal level for 5 yr. At MTL the reduction in *Chthamalus* coincided with maximum development of the *Fucus* canopy in 1970, but although all the large algae had gone by 1973 the numbers of *Chthamalus* at this level are still less than those found over the period 1956–66, possibly an effect of the increased *Patella* population. At MLWN the barnacles were smothered by the upward extension of the infralittoral fringe (see below) and *Chthamalus* returned gradually over a period of 5 yr, and is now just within its former range of abundance after a peak in 1973.

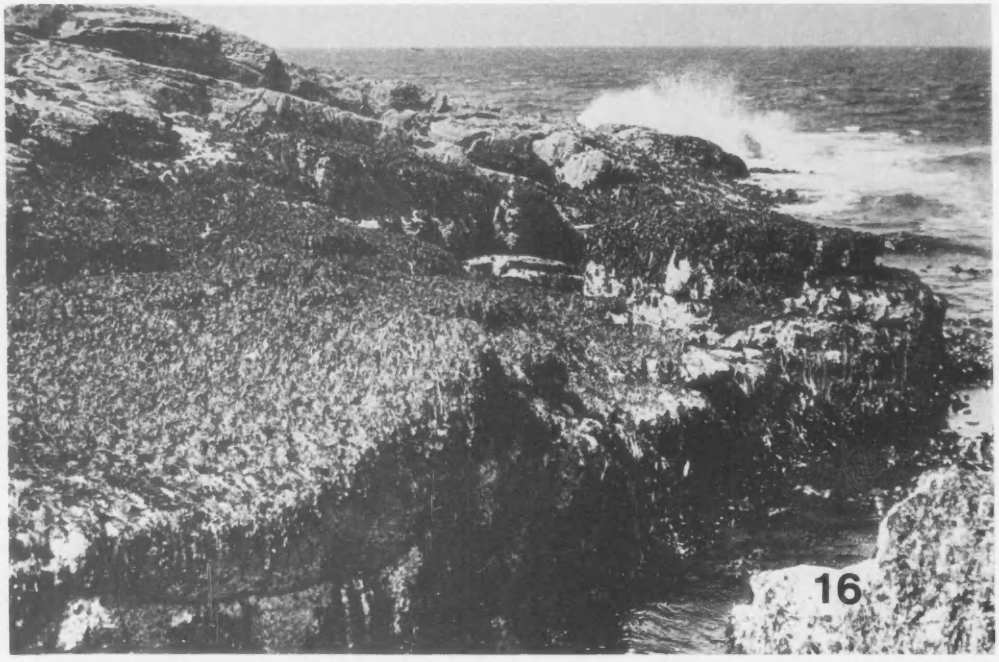
Although *Patella* was wiped out at Cape Cornwall in 1967, *L. nigrolineata* Gray survived. This species is apparently oviparous (Bergerard 1971; Heller 1975) and is usually found at HWS–HWN in association with *Chthamalus*. The survivors showed the effects of the cleaning operations in the same way as the surviving *Nucella* at Porthleven (Bryan 1969) in the form of a growth check on the shell. The numbers increased, and in 1970 and 1971 they could be found aggregated at the base of the clumps of *Fucus*, together with *Patella*, and probably contributed to the eventual removal of the algae. The year 1970 marked the return of other herbivores, *G. umbilicalis* and *L. obtusata*, both in slight shelter, the latter taking advantage of the greater *Fucus* cover than normal to extend over towards the wave-beaten outer edge of the reef. A few young specimens of *Nucella* were found at Cape Cornwall in 1968, and it is assumed that one or two adults survived to breed or else some egg cases survived the cleaning operations as at Porthleven. By 1972 the population of this species had increased considerably, with up to 15/m² on vertical faces on the sheltered side of the reef. The large top-shell *Monodonta* took longer to return here, and was not seen until 1972, when a few *L. rudis* Maton were also recorded.

The inner side of the reef studied at Cape Cornwall, like the rest of Porthledden, of which it is the outer part, retained a full "green flush" into 1968, unlike the wave-beaten outer edge where *Fucus* built up during 1967–68. *Fucus* did not dominate Porthledden until 1969, and it seems possible that the delay may reflect the heavy dispersant treatment received in the inner parts of the bay.

At Sennen Cove there was a similar prolongation of the "greening" and delay in the subsequent development of *Fucus* cover, in an area extending from 100 m west of the pier eastwards to the main sandy beach. The extremely heavy oiling here was treated for a long time with dispersants, and as we know that the oil persisted in the sands for at least a year, it seems possible some components of the dispersant also lingered in the sands and in sand and gravel under the boulders for a shorter period. In this region no molluscs survived to 1968. In 1970 *N. lapillus* and *G. umbilicalis* were occasionally found, and by 1972 all the top-shell species had returned, together with *L. rudis*. Our routine survey station at Sennen is 300 m west of the pier and received less dispersant (Fig. 16, 17). As at Cape Cornwall, *L. nigrolineata* survived when the limpets were killed, and *Fucus* cover developed during the 1st yr, the remaining changes being quite similar to those on the outer edge of the reef at Cape Cornwall (Table 2).

### MAEN DU POINT, PERRANUTHNOE

We have already mentioned the dispersant treatment applied to Perran Sands at Perranuthnoe, and the effects on the beach infauna have been reported in Smith (1968). The rocks at the northwest end of the beach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received moderate oiling, comparable to Godrevy Pt., but up to the beginning of May 1967 many herbivores, including the top-shells, were still alive, though some limpets had died in HW pools close to the thicker patches of oil stranded at extreme high water spring tide level (EHWS).

Oil was still present on these rocks a year later, hence we think that most of the effects then found are due to drift of oil-dispersant patches from the beach operations. This drifting was seen from the cliff top in 1967. At any rate, the observations in 1968 show that most of the limpets had eventually died, though some of the *Monodonta* survived. Much "greening" was then present, together with heavy settlements of *F. vesiculosus*. A slight trace of oil persisted at EHWS to 1970, but by this time it had weathered and was no worse than the more usual small patches of "chronic" oiling that occur afresh every year on most Cornish shores. The maximum cover by *Fucus* varied from 50% (*F. spiralis* at HWS) to 80% (*F. vesiculosus* at MTL–LWN), and by 1970 *P. vulgata* and *P. depressa* had returned in some number. In later years, in spite of the lower mortality that had occurred among the herbivores, and the lesser density of the *Fucus* cover, the changes took as long as on shores treated directly with dispersants (Table 2).

### Godrevy Point

Moderate oiling occurred at Godrevy Pt., but this is National Trust owned land, and lies close to a well-known breeding ground of the Atlantic gray seal. In consequence, direct dispersant treatment was resisted. However, there may have been drift from nearby parts of St. Ives Bay, and the oil itself may have carried traces of the persistent fractions from dispersant spraying at sea a week before stranding. It is thus not possible to be sure, but from circumstantial evidence we believe this is the only station we inspected where the effects can be attributed to the oil. The oil was principally restricted to the inner parts of the reef, from MTL to EHWS, but was nowhere more than 1 to 2 mm thick; it remained in the form of a water-in-oil emulsion for some weeks in spite of exposure to sun and air at low tide. After a month some of the barnacles had managed to clear an opening in the film of oil and showed normal cirral activity when examined in the laboratory. The gut did not contain any traces of oil, in marked contrast to grazing herbivores examined at the same time. The mussels *Mytilus edulis* were apparently unharmed, and we saw no change in the *Nucella* population. Limpets, *P. vulgata*, however, suffered heavily on oiled vertical faces between MTL and MHWS. The limpets must have died, or been weakened sufficiently to lose hold of the rock, or else they moved elsewhere, for their "scars" or homes, where they had lived before the disaster, showed up as light-colored patches against the blackish-brown coating of oil. On flatter surfaces at Godrevy, as on other lightly treated shores, we found limpets browsing on oil-covered rocks, and oil droplets were present in the gut. At this stage of weathering the oil ingested did not appear to be toxic, and a few limpets that had actually grown and increased their shell-size on a diet including oil were found later on another shore.

In spite of the apparent loss of limpets there was no abnormal "greening" of this shore, which often carries patches of *Entermorpha* and *Porphyra* during the spring. By 1968 some limpets had recolonized the vertical faces affected in 1967 though in lesser numbers than before. Many clear patches of rock could be seen, however, and it was obvious that about 50% of the barnacles had died. Traces of oil remained on flatter surfaces in 1968, but a new settlement of *B. balanoides* took place in the spring, regardless of this remaining oil, and *Chthamalus* also resettled later in the year.

All the oil had gone by 1969, and no obvious long-term effects could then be seen. The mortality at this place was therefore confined to barnacles and limpets that had been thickly coated with oil, and a large section of the shore was apparently unharmed. The whole reef was back to normal after 2 yr, in striking contrast to those shores that were treated with dispersants. The oil did kill some animals, and was thus not as harmless as we thought in 1967 (Smith 1968), but it was obviously much less toxic than many other crude oils and distilled products (Blumer et al. 1970; North et al. 1965; Sanders 1973; Thomas 1973; Kuhnhold 1974; Linden 1976; van Gelder Ottway 1976; Neff et al. 1976; Rice et al. 1976).

### Shifts in Zonation Patterns

A most interesting aspect of the process of recolonization of the denuded shores was a change in zonation patterns resulting from alterations in species interactions and the differing time scales of resettlement.

Such a change was most obvious on the outer edge of the reef at Cape Cornwall (Fig. 18), where there was an especially luxuriant regrowth of algae by the spring of 1968. On the traverse investigated, the infralittoral

FIGURES 16 AND 17. Sennen Cove, wave-beaten rocks at the south end.

FIG. 16. May 21, 1970. Near the camera there is dense cover by *F. spiralis* (*upper edge*) and *F. vesiculosus* f. *linearis*, but farther to seaward the algal cover is already thinning out as the new population of herbivores develops. Fig. 17. June 1, 1977. A more or less complete return to normal *Patella/Chthamalus* dominance has occurred. There are a few tufts of *F. vesiculosus* in the barnacle zone (as immediate foreground), and the black lichen *Lichina pygmaea* has recovered its former abundance at high-water (*upper left side*).

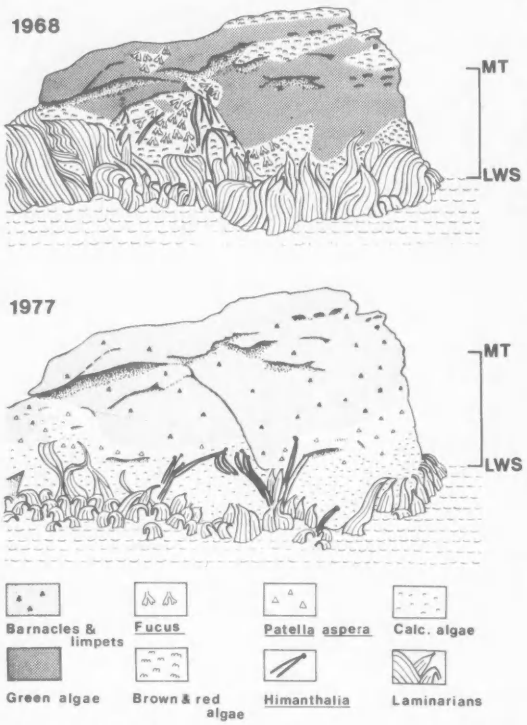


FIG. 18. Cape Cornwall, wave-beaten outer rocks: sketches based on photographs showing the change in zonation after the disaster. *Upper*, situation in May 1968, 13 mo after all herbivores had been killed by dispersants; below, situation 9 yr later in May 1977, after restoration of the herbivore population. Upper limits of *Laminaria digitata* and *Himanthalia elongata* were 1.5 to 2 m higher in spring 1968 than in spring 1977. MT = mean tide level, LWS = mean low water spring tide level.

fringe (principally *L. digitata*, though *Alaria esculenta* and *L. hyperborea* are present), was raised above its normal upper limit by 1.5–2 m, so that quite large plants of *L. digitata* were growing at between MLWN and MTL in places where there was previously a community of barnacles, limpets, and smaller algae. A corresponding upward shift had taken place in *Himanthalia elongata*, which at this place normally forms a belt overlapping the infralittoral fringe and the lower third of the midlittoral. Plants with well-developed "thongs" were present up to MTL, and "buttons" occurred right up to MHWN. In addition *Corallina* was much better developed and bushier than usual. There was dense cover by young plants of *F. vesiculosus* f. *linearis* from MHWN to MTL, and some patches of this extended right down to MLWN, mixed with the *Himanthalia* and *Laminaria*. After 3 yr the *Laminaria* had retreated, but *Himanthalia* was still present in a thick belt at least 1 m above its usual level, and a return to normal patterns was not seen until 1973–74, after the peak in the limpet population had been reached.

This upward shift in the algal zonation must be related to the absence of grazing pressure by limpets. On such a wave-washed site it would appear that this biological interaction may be more important than a physical factor such as desiccation, which has usually been accepted as the cause of the upper limit of the infralittoral fringe. This hypothesis probably still applies to sheltered shores where *P. aspera* is absent and limpets less common. At wave-beaten edges of the reef at Cape Cornwall *P. aspera* is normally dominant in the region showing the change in zonation, and occurs in smaller numbers right up to MHWN. This species recolonized between MLWN and MTL in small numbers in 1967–68, and had reached a length of 9 to 17 mm by the spring of 1969. These measurements indicate a slower rate of growth than in *P. vulgata* and probably a slower build-up in the population, hence the restoration of the normal algal zonation at this level must be attributed also to the larger number of *P. vulgata* present at MTL and their subsequent movement down the rock. *P. aspera* increased later, and the species persisted in smaller numbers at MTL to MHWN for five years after the end of the *Fucus* canopy.

Other changes in zonation have occurred, less obvious than the shift in algae, but involving similar vertical distances. Most of them were upward extensions of species which took advantage of the shelter and increased moisture provided by the *Fucus* cover. Thus, in 1969 on several shores, including Lizard Pt., Porthleven, Perranuthnoe, Sennen Cove and Trevone, *S. rupestris* became abundant at MTL under the canopy of *F. vesiculosus* and *F. serratus*. In such places it was replacing the normal barnacle zone (cf. Moore 1934), and was frequently found in association with other organisms more usually found towards low tide level or in pools, including encrusting coralline algae ('lithothamnia'), the barnacles *B. crenatus* and *B. perforatus* and the LW limpet *P. aspera*. *Spirorbis rupestris* was not confined to "lithothamnia" coated surfaces, and also occurred on the bare rock and on limpet shells. The uplift of *B. perforatus* was particularly noticeable at Porthleven and on the outer edge of the reefs at Trevone. Some specimens occurred up to MHWN where they survived for a year or two after the end of the *Fucus* cover.

During the period of *Fucus* decline, especially after 1972, it was quite easy to distinguish places where the algae had been abundant by the presence of the gradually dying remains of all of these uplifted animal zones, and by patches of "lithothamnia." The general abundance of the crustose coralline algae was noted on all *Torrey Canyon* shores, but some of the growth of these species could be related to the initial absence of limpets as well as to the greater moisture levels under the *Fucus*. Existing limpet "fronts" show clearly the heavy grazing pressure also exerted on such calcareous algae.

It would seem therefore that the disturbance of the ecosystem caused by removal of all grazing animals had two effects on zonation; one was related to a lessening of desiccation caused by algal growths; the other was more complex, and resulted from direct interaction between the herbivores and the plants. It seems possible that corresponding changes have occurred elsewhere through oil pollution, even when dispersants have not been used (cf. Thomas 1975).

### Loss of Species

The absence of evidence showing loss of a species has often been claimed to show that the dispersants had no long-term effects on the ecosystem (e.g. Shelton 1971; Wardley Smith 1976). We have no data for the algae, which are still in need of a complete survey. However, one animal species has not returned to shores directly treated with dispersants: the rare, warmwater, hermit crab, *Clibanarius erythropus* (Southward and Southward 1977).

The British population of *Clibanarius* appears to be a precarious one, maintained by occasional settlements of planktonic larvae derived from the more abundant populations in south Brittany, and it is possible that recent climatic and hydrographic changes may have reduced the chances of recruitment.

There is a surviving colony of this species at Wembury, South Devon, outside the affected area, and a few specimens escaped at Great Hogus rock, Marazion, when others were killed on the causeway rocks. But at Lizard Point, Porthleven, and Trevone the species was wiped out and has not returned; the latter locality was the only known record on the north coast of Cornwall.

Strictly speaking this species has not been totally lost from the Cornish fauna, but the known population in Britain has been reduced by half from that existing before the *Torrey Canyon* disaster. We could regard this as the $LC_{50}$ for the species.

## Discussion

The widespread mortalities on west Cornish seashores resulting from the *Torrey Canyon* clean up provided a large-scale experiment on the successional sequence of rocky shores and on the influence of herbivores and predators on the ecosystem. The small amount of prespill data, the lack of sites where oil was left entirely untreated, and uncertainties of how much dispersant reached marginal areas have prevented accurate statistical comparisons. However, the frequent long stretches of coastline showing dispersant damage has meant that the first few years of recolonization relied more on larval dispersal than on local adult migration, a factor that has always interfered with small-scale experiments on rocky shores (cf. Southward 1956; Aitken 1962; Emson and Faller-Fritsch 1976). Incomplete as the results are, we still have a mass of information which helps to illuminate current theories on the structure and stability of ecosystems.

Reference to almost any recent ecological journal or book will show the continuing lack of agreement on what constitutes stability, and how it is related to diversity (Odum 1971, 1975; Orians 1975; May 1975; Menge and Sutherland 1976; McNaughton 1977). On rocky shores environmental stress by physical factors can be important (Sanders 1968; Lewis and Bowman 1975; Bowman and Lewis 1977) but biological interactions appear to provide many of the reasons for the community patterns observed (e.g. Connell 1972, 1975). In a simple system with high environmental stress (Menge 1976) interspecific competition for space is important in wave-exposed areas, predation important in shelter. In more complex communities both predation and competition for space and food operate in different facets of the community or at different tidal levels (e.g. Connell 1970; Luckens 1975; Branch 1976; Menge and Sutherland 1976). However, experimental removal of a dominant predator or grazer results in an increase in biomass, and often reduces species diversity (Paine 1966, 1971; Paine and Vadas 1969; cf. Connell and Orias 1964; McNaughton 1977).

It should thus be no surprise that a disturbance on the scale of the *Torrey Canyon* affair can cause long-term instabilities to a typical northeast Atlantic rocky shore community, and shorter-term changes in zonation patterns. It was at one time thought that the typical shore of the region was dominated by belts of the large brown seaweeds, and that the transition to a barnacle- or mussel-covered shore in exposed points was a direct effect of the wave on settlement and survival of the plants, and there was some experimental evidence to support this view (Hatton 1938). However, experiments carried out since 1946 show that the negative correlation between wave action and a *Fucus*-dominated community is indirect, mediated through the grazing activities of herbivores, mainly limpets (Jones 1948; Lodge 1948; Burrows and Lodge 1950; Southward 1956; Aitken 1962). Removal of the limpets from a west coast shore of Britain produces a regular succession: (1) a film of benthic diatoms and other unicellular and filamentous algae, a brief transitional phase that may be obvious only in late autumn and winter when lack of light reduces algal growth; (2) a green flush of *Enteromorpha*, which may dominate for a year; (3) heavy settlement of *Fucus* in the 1st–3rd yr, replacing the *Enteromorpha*; (4) settlement and growth of *Patella* follows, so that further increases in *Fucus* are prevented by grazing during and after the 3rd yr; (5) the older plants then die off or are removed by *Patella* grazing; (6) the rock is available for resettlement by barnacles in the 5th and 6th yr, and the number of limpets falls as their food supply is reduced; (7) by the 6th and 7th yr or so there is a return to a *Patella*–barnacle dominated community, and the cycle could be restarted again. At the end of the field experiments quoted above, the cleared areas could be distinguished from the surrounding natural communities by a total absence of large algae above MTL.

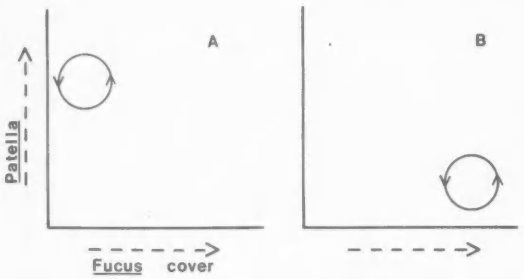


FIG. 19. Model of the theoretical relationship (Lotka-Volterra plot) between the numbers of a herbivore (*Patella*) and the density of an alga (*Fucus*). A, On wave-beaten shores; B, on sheltered shores.

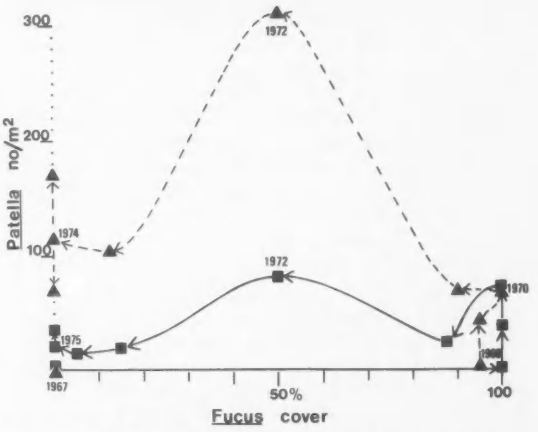


FIG. 20. Number/m² of *Patella* plotted against the percentage cover of *Fucus* at MTL Trevone (solid line and squares) and at MTL Porthleven (broken line and triangles) to compare with Fig. 9. Dates of some of the points in the sequence are shown.

Natural cycles with the same succession occur on small scale, due to storms, beach movements, or similar factors causing local reduction in the limpet population, and may follow shipwreck or harbor construction where new surfaces are exposed to the sea (Seshappa 1956; Southward 1956; Moore 1939). Not all of the steps in this succession necessarily follow the preceding stage, in agreement with Connell (1972). Barnacles can settle immediately on cleared surfaces at some seasons (personal observations), and limpets can follow *Enteromorpha* without a *Fucus* phase, but most often the full sequence is seen. The same cycle of events was followed on the shores badly affected by the *Torrey Canyon* clean up, but the situation was complicated by the fact that all herbivores and some of the barnacles were killed. In the absence of *Patella* the littorinids can exercise control over algal regrowth (cf. Menge 1976; Keser et al. 1977), while dead barnacle shells would be expected to provide a surface favourable to settlement of spores (cf. Menge 1976). Nevertheless, the main course of recolonization has been similar, and, as on the experimental areas, there have been fluctuations in zonation patterns and the sequence has ended with a shore devoid of large seaweeds (Burrows and Lodge 1950; Southward 1956). Only the time scale has been different. Along the wave-beaten edges to the reefs and in a few spots where *Fucus* failed to become dominant the cycle was completed in 5–7 yr; elsewhere periods of up to 10 yr have been noted. This longer period to regain equilibrium may be due to (1) residual toxicity, with delays up to 1 yr, (2) the sheer scale of the mortality restricting possible migration of adult herbivores such as was possible on small experimental areas and must happen naturally, (3) the removal of all herbivores, as noted above. If a future major spill of toxic oil should occur, more attention must be paid to these aspects of recolonization, by sampling for oil and dispersant residues, and by examining the edges of the treated areas for migration.

In the absence of large-scale disturbances and where environmental fluctuations are of small amplitude, the balance between the large seaweeds and the herbivores either becomes stabilized at some particular point in the cycle, or undergoes cyclic changes of smaller radius (Fig. 19). Increasing wave action favors the limpets and barnacles while increasing shelter and increasing shade favor the algae, but in a natural system the two extremes — all algae, or all barnacles and limpets — are rarely found. Instead there is usually a mosaic related to small-scale local variations in wave action, insolation, desiccation, herbivore density, and irregular predation by the next trophic level (*Nucella*, crabs, fish, and birds). We are here considering a very simplified version of the system, and have not taken into account other common animals such as mussels (Lewis 1972, 1976), the pseudoperennial algae such as *Laurencia* and *Corallina*, and the smaller and encrusting algae which are also grazed by the herbivores. Even with these additional factors, the situation is still simpler than in other parts of the world where limpets are more specialized, and more species exist in closer relationship with their food organisms (Branch 1976).

We can consider the fluctuations in algae, herbivores, other sedentary animals and predators resulting from the *Torrey Canyon* affair as a series of damped oscillations in which the delay in response of one organism to changes in the other is much greater than in a typical Lotka-Volterra system (cf. Odum 1971). The time scale of the oscillations is prolonged since breeding and recruitment are annual (or less frequent) and the typical organisms can live for a long time: *Fucus* 4–5 yr (Knight and Parke 1950), *Patella* 20 yr (Fischer-Piette 1941), *Chthamalus* 5 yr. The point at which the limpet–*Fucus* balance reaches equilibrium can still be illustrated in terms of Lotka-Volterra equations, though this is a great oversimplication (cf. Harte and Levy 1975). By plotting the changes on *Torrey Canyon* affected

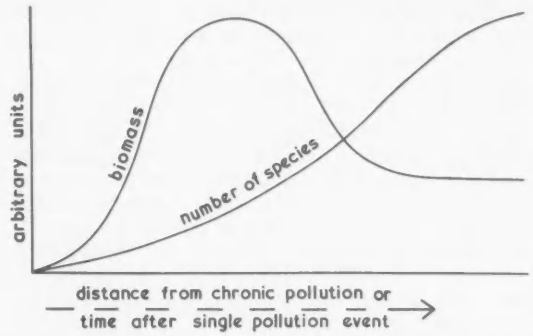


FIG. 21. Theoretical representation of the effects of disturbance to the ecosystem on rocky shores. The ordinate scale can indicate distance from a source of chronic pollution, or (as in the *Torrey Canyon* sequence) the time from a single catastrophe.

shores compared with the model (Figs. 19–20) we can see that the relationship swung first to a part-cycle representing the more sheltered shore type of community, then to a part-cycle typical of more exposed shores, but eventually the system runs off the graph since the food supply is drastically reduced. The relationship between *Patella* and *Fucus* is thus confirmed to be much less of a specialized "prey"–"predator" system than that found between the kelp *Egregia* and the limpet *Acmaea insessa* (Black 1976) and some of the South African species (Branch 1976). *Patella* can and does graze on many other food organisms, and is also capable of modifying its grazing pressure by extensive migration (Aitken 1962) where local variations exist.

In theory the balance of the intertidal rocky shore community could be disturbed (cf. Cowell 1976) by any man-made event that alters the abundance of one of the common species. It is most often the herbivores that are sensitive, whether to oil, dispersants, sewage, or other toxins, and hence the effects are usually seen as a transition, temporary or permanent, towards the sheltered shore type of community (Baker 1976a). Such a shift in the equilibrium reduces the number of species, though the total standing crop (biomass) may be higher. We may express the relationship as a general principle (Fig. 21), based on the model suggested for polluted rivers (e.g. Hynes 1950). Apologists for pollution, and some fishery technologists, like to think of the lower diversity mode of such a system as an increase in productivity (cf. Regier and Cowell 1972; Cowell 1976). In the intertidal habitat we must be careful to separate increases in biomass, as in the growth of perennial seaweeds, from the lesser annual primary production.

A final point to be considered in relation to the time scale of recovery is climate. This is one of the factors that may have helped to mitigate some of the effects of the *Torrey Canyon* on marine life in Cornwall, though it may have contributed to the mortality of certain species (cf. Crapp 1971a). Cornwall is close to a boundary between zoogeographical regions. It is at the southern limit for many boreal species, but temperatures are high enough to permit some warm-temperate species to live and reproduce. In colder waters we would expect the oil to weather much more slowly and there would be slower recolonization by the organisms affected (Prouse and Gordon 1976; Vandermeulen et al. 1977). On the other hand, in low latitudes blackening by persistent oil can lead to very high temperatures in the intertidal zone due to absorption of solar radiation (Chan 1977a; cf. also Straughan 1976) and this could delay recolonization even though the progress of weathering would be faster and the period of settlement greater in the tropics (cf. Stirling 1977). On Cornish shores the oil weathered quite rapidly when it was given the chance, and resettlement was not noticeably influenced, giving us the best of the two extremes. However, the position at the edge of distribution of certain species has contributed to the partial loss of a rare animal and may have been one of the causes of the delay in recruitment of southern species such as *P. depressa* and *Monodonta lineata*.

## Conclusions

It is unfortunate, to say the least, that the myth of rapid and complete recovery of *Torrey Canyon* affected shores has become enshrined in the literature, and has even reached elementary textbooks on pollution ecology (e.g. Mellanby 1972). We must emphasize again, therefore, that recovery has not been rapid. Recolonization, first by algae and then by animals has taken place slowly year by year, the number of returning species increasing as dominance by opportunists and early colonizers became reduced. Many of the species breed for a short time only once a year, and not all of the animals have planktonic larvae. Return to normal has therefore been very protracted. As might have been expected, the lightly oiled wave-beaten rocks that received moderate dispersant treatment have shown the most complete return to normal, taking about 5–8 yr. Other places that received the brunt of dispersant treatment have taken longer, from 9 to 10 yr, and it is the opinion of other ecologists besides ourselves that some shores are still not normal. This is a partly subjective estimate, based not only on species richness but on the presence of a wide range of sizes and age-groups of the longer lived species and on the "photographable" quality of the mosaic of contrasting organisms that characterizes a mature and balanced intertidal community on the open coast of Cornwall.

The effects have been more obvious, and have probably been detectable for longer periods on the west coast of Britain than they might have been in places where limpets are less abundant or in regions where the genus *Patella* is absent (cf. Stephenson and Stephenson 1972). However, in some regions where limpets are less dominant there is a more complex pyramid of

herbivores and several levels of predation (e.g. Pacific North America; Connell 1970; Menge and Sutherland 1976), and the aftereffects of oil spills can be of still greater severity (North et al. 1965; North 1973; but contrast Chan 1977b). It is perhaps the one tangible benefit of the *Torrey Canyon* affair that it drew universal attention to the dangers of dispersant. Thus our misfortune in Cornwall has helped prevent excessive use of dispersants to clean up later spills such as the Santa Barbara blowout and the *Arrow* wrecking (Straughan 1971; Canada, Ministry of Transport 1970), and has eventually lead to some changes in U.K. official policy with regard to treatment of spills.

In discussing the use of dispersants to clean the shores affected by the *Torrey Canyon*, the editor of the Marine Biological Association report (Smith 1968) says (p. 178) "the decision to use detergent for the dispersal of the oil was taken on the view — with which there will be general agreement — that the preservation of the coastal amenities was of first priority, and in the hope that the effects of the detergents on marine life would not be catastrophic." This view has been applauded and amplified beyond the original in a review by Mackin (1973). The suggestion that there was no alternative approach to the problem than widespread dispersant application is not borne out by experience in Guernsey and France and by later spills. It is quite clear now, with hindsight, that the developers of the dispersant technique never thought it would be used on a massive scale. Thus, Wardley Smith (1962) believed that only large local authorities, with considerable tourist interests, would be able to afford the cost of cleaning up their beaches, and urged more measures to prevent spills. But in Cornwall, in 1967, cleaning operations soon got out of control, with dispersants issued in virtually unlimited quantities and the armed forces called in for assistance. In Guernsey there was no help from the military, and as dispersant was fully costed only a small quantity was used; the effects on marine life were minimal, but the tourist interests were unharmed. In Brittany use of dispersants was also restricted, though more oil was stranded there than in Cornwall, yet tourist interests were again unharmed.

Much depends on ones view of what constitutes recreational amenities. The House of Commons Select Committee on Science and Technology (Great Britain, House of Commons, 1968) took the view that "to many people one of the particular delights of visiting the seaside is to study and enjoy the vast variety of the fauna and flora." Cowell et al. (1972) endorse this and note "shores that were cleaned in order to render them suitable for holiday makers are now very difficult to travel over and will remain so for several years." That is, a layer of oil that might have degraded within a year was exchanged for slippery thickets of one or two species of seaweed of much greater persistence. It is obviously more important to consider amenity use when oil is stranded on sandy shores close to tourist centers, but even then, during the *Torrey Canyon* operations dispersants were used in such large quantities that some of the oil was driven deep down into the sands and only released later during winter storms. Similar consequences of dispersant application to sandy beaches are still being reported after recent oil spills, such as the *Jakob Maersk* in Portugal (Duerden 1976) and the *St. Peter* in Colombia (Hayes 1977; D. J. Crisp personal communication), showing how little seems to be learned at the practical level. It is only now, 10 yr later, after studying the results of other strandings and of laboratory experiments that one can see how fortunate we were in Britain that the *Torrey Canyon* disaster was no worse. If the wreck had happened on a more embayed section of the coast or in an estuary complex, if the tanker had been carrying distilled products or one of the more toxic crude oils, if all the oil had come ashore and had been treated with an equivalent ratio of dispersants, in these cases we might indeed have been closer to ecodisaster.

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# Comparison of Oiled and Unoiled Intertidal Communities in Chedabucto Bay, Nova Scotia[1]

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THOMAS, M. L. H. 1978. Comparison of oiled and unoiled intertidal communities in Chedabucto Bay, Nova Scotia. J. Fish. Res. Board Can. 35: 707–716.

During 1976, detailed surveys of four oiled and four unoiled control stations, each subdivided into seven standardized intertidal levels, were carried out in Chedabucto Bay. Seventy-one species were found, 14 unique to control and 9 to oiled locations. Species diversity was uniformly higher at control than oiled stations. No differences in horizontal zonation of major species were apparent. Analysis of abundance and biomass data for the eight stations and seven tidal levels showed a significant overall difference between oiled and control situations. However, no particular station or tidal level was significantly different from any other. Ten species accounted for most of the variance between oiled and control stations. Six of these were more important at controls and four more important at oiled stations. The flora were particularly affected at oiled stations and species dominant on both sedimentary and rocky shores at all but the lowest tidal levels have been reduced. Length and weight data for the clam *Mya arenaria* showed significantly lower values at oiled stations, but that for the periwinkle *Littorina littorea* showed the opposite. The length–weight relationship for both of these species showed a significantly lower increase in weight per unit of length at oiled than at control stations. Oiled stations showed significantly greater concentrations of oil in biota and sediments than unoiled, where concentrations were essentially at background levels.

*Key words:* hydrocarbon, intertidal community, petroleum, pollution, sediment

THOMAS, M. L. H. 1978. Comparison of oiled and unoiled intertidal communities in Chedabucto Bay, Nova Scotia. J. Fish. Res. Board Can. 35: 707–716.

Au cours de 1976, nous avons effectué des relevés détaillés de quatre stations affectées par le pétrole et de quatre stations témoins non touchées par le pétrole dans la baie Chedabouctou. Chaque station a été divisée en niveaux intertidaux standardisés pouvant aller jusqu'à sept. Nous avons trouvé 71 espèces, 14 particulières aux stations témoins et 9 aux stations affectées par le pétrole. La diversité des espèces est toujours plus élevée aux stations témoins qu'aux stations affectées par le pétrole. Il n'y a pas de différences apparentes dans la zonation horizontale des principales espèces. L'analyse des données sur l'abondance et la biomasse aux huit stations et aux sept niveaux de marée révèle une différence significative dans l'ensemble entre les situations où il y a pétrole et les témoins. Cependant, aucune station ou niveau de marée ne diffère significativement des autres. Dix espèces sont responsables de la plus grande partie de la variance entre les deux groupes de stations. De ces espèces, il en est six qui sont plus importantes aux stations témoins et quatre plus importantes aux stations touchées par le pétrole. La flore est particulièrement affectée aux stations où il y a pétrole et les espèces dominantes sur les rivages sédimentaires aussi bien que rocheux ont été réduites à tous les niveaux de marée, sauf le plus bas. Les données sur la longueur et le poids de la mye commune, *Mya arenaria*, ont des valeurs nettement inférieures aux stations touchées par le pétrole, alors que celles du bigorneau comestible, *Littorina littorea*, démontrent le contraire. La relation longueur-poids de ces deux espèces accuse une augmentation nettement inférieure dans le poids par unité de longueur aux stations touchées par le pétrole par rapport aux contrôles. Les stations affectées par le pétrole ont des concentrations nettement supérieures de pétrole dans la biocoenose et les sédiments aux stations non touchées par le pétrole, où les concentrations sont essentiellement à des niveaux de fond.

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DESPITE the now large number of oil spills there are still comparatively few published accounts of long-term effects on intertidal biota of the oil alone. Where studies have been carried out, most have involved various types of crude and refined oils (Baker 1976; Hampson and Moul 1978; Southward and Southward 1978; Straughan 1977; Straughan et al. 1978) but not Bunker C. Few studies have been carried out in cold climates.

In February 1970 the tanker *Arrow*, loaded with Bunker C oil, grounded on Cerberus Rock in Chedabucto Bay, Nova Scotia. Large quantities of oil escaped and extensive areas of shoreline in the area were contaminated (Anon. 1970). Since that time the distribution and abundance of oil and common intertidal biota have been monitored at a series of seven stations, situated on a variety of shore types on Isle Madame in the northern portion of the Bay.

This experience has provided a long series of observations on a variety of shores, in a cold climate, that were heavily contaminated by Bunker C oil. It should be realized, however, that long-weathered crude and Bunker C residues would be similar because only similar high-boiling point fractions and insoluble compounds would remain. The result of these surveys have been published by Thomas (1972, 1973, 1975, 1977) and Thomas and Harley (1973).

In summary, it was demonstrated that oil adhered strongly to the upper two thirds of the intertidal zone being most persistent at about mean high tide level. After 1970 surface oil declined at varying rates. Attrition was most rapid at lower tidal levels on exposed shores and slowest at high tide levels in sheltered locations. Exposed rocky shores were generally free of surface oil after 3 yr, but considerable oil still persisted at high tide level in sheltered lagoons, 7 yr after initial oiling.

Biotic effects were documented in a number of common species. On rocky shores, where fucoid seaweeds are dominant, the intertidal range of *Fucus vesiculosus* was depressed down the shore, followed by a slow return to normal distribution by 1975. *Fucus spiralis*, which is normally confined to a narrow zone at about mean high tide level, disappeared at study sites and had not reappeared by 1976. By contrast, populations of abundant shore fauna including barnacles, *Balanus balanoides*, and periwinkles, *Littorina littorea*, *L. saxatilis*, and *L. obtusata* did not show changes in distribution and/or abundance except where algal changes affected their habitat.

In sheltered locations, shores are of sand or mud and there, salt marsh cord grass (*Spartina alterniflora*), common at upper intertidal levels, suffered heavy mortalities delayed 1 yr from the initial spill. Recovery was evident 2 yr later and populations appeared essentially normal in 1975. At lower levels on these shores, the soft-shell clam *Mya arenaria* was abundant. This species suffered heavy mortalities where oil cover was extensive but has shown consistent annual recruitment. Mortalities declined with oil but were still above normal in one location during 1976.

These extensive mortalities involving several dominant intertidal species over a long time period suggested that persistent widespread ecological changes had occurred and that a thorough study of the situation was warranted. In 1976 a detailed study was made of biotic communities and oil distribution at four of the initial seven study locations and these were compared to the situation at four unoiled but essentially similar locations in the same general area.

## Materials and Methods

### STUDY SITES

Figure 1 shows the locations of the four oiled and four control sites all of which were situated on Isle Madame. Table 1 lists locations and shore types for the four pairs.

Pairs of stations were selected to be similar, as far as possible, in (1) exposure to wind and sea, (2) substrate type and distribution, (3) a similar shore slope, and (4) biotic characteristics.

### SURVEYING AND SUBSTATION SELECTION

At each station a transect, to act as a reference in distributional studies and a central line in population studies, was selected to run a right angle to the mean water line. Heights of all points along this transect were determined with reference to mean low water using the method described by Thomas (1974). The intertidal distribution of

TABLE 1. Characteristics of study locations.

| Oiled | | Control | | |
|---|---|---|---|---|
| Station location | Width[a] | Location | Width | Substrate |
| 1. Petit Barachois | 14 | Bewes Pond | 8 | Bedrock HW |
| 2. Janvrin Lagoon | 70 | Potato Island | 30 | Muddy sand HW<br>Sandy mud LW |
| 3. Crichton Island | 21 | Beak Point | 65 | Mainly bedrock<br>Some sand HW |
| 4. Janvrin Lagoon | 25 | Potato Island | 21 | Muddy sand HW<br>Sandy mud LW |

[a]Horizontal distance in metres, high water level (HW) to low water level (LW).

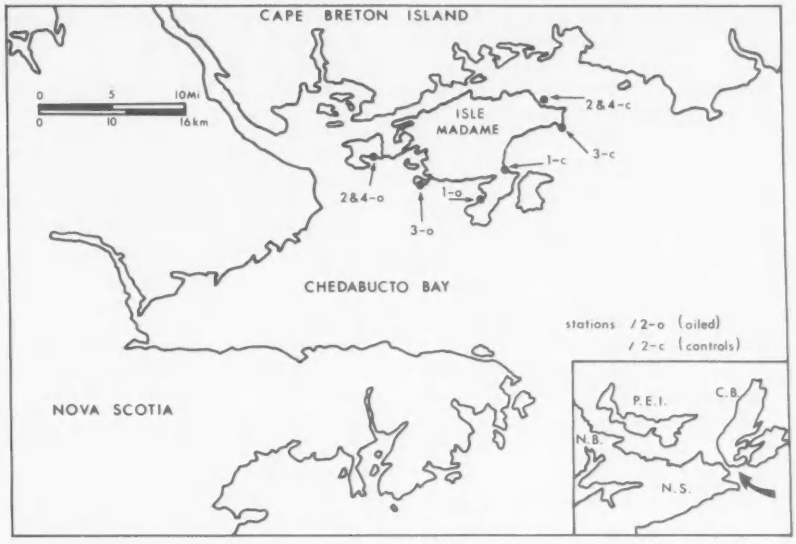


FIG. 1. Chedabucto Bay area of Nova Scotia showing locations of oiled and control study stations.

all shore biota were determined in a belt extending 0.5 m on both sides of the line.

Substations for quantitative studies of biotic communities were established on the line at the following tidal levels where possible: −0.3 m, 0.0 m (mean low water), 0.3 m, 0.65 m (mean tide level), 1.0 m, 1.3 m (mean high water), and 1.9 m (extremely high water). Some levels were not available for study at all locations due to local topography.

### COMMUNITY STUDY METHODS

At each substation the biotic community was described by removing all biota within a quadrat of 0.1 to 1.25 $m^2$ (mean 0.43 $m^2$, $n = 37$). The size of the quadrat was adjusted to give a total sample of about 1 kg fresh weight except where biota were sparse. In sedimentary locations sediment was screened through 4-mm mesh to a depth of 10 cm. From these collections, biomass was determined for each species on a fresh weight basis and the number of individuals of each species was counted. Samples were then frozen at −20°C and later dry weight and ash free dry weight biomass for each species were determined. All statistics were adjusted to represent samples of 1 $m^2$.

Outside the quadrat but at the same tidal level, samples of 50 *L. littorea*, 50 *M. arenaria*, and/or 1 kg of *S. alterniflora*, whichever were present, were collected at random for measurement of length and weight of individual fauna and for oil content analysis of all species.

### OIL SAMPLE COLLECTION AND SURFACE OIL MEASUREMENT METHODS

In sedimentary locations cores of 5.5 cm diam and at least 10 cm long were taken by hand corer pushed vertically into the sediment to a natural stop. These samples were deep frozen at −20°C for later analysis. On rock, the percentage oil cover was measured using various sized quadrats. All sample locations were photographed.

### OTHER METHODS

At substations at station 2-oiled, where *M. arenaria* mortalities had previously been measured (Thomas 1977), mortalities were again estimated. A core for oil analysis was taken at substation 2 where oil was still visible; samples of living and freshly dead clams (bodies present in shells) were collected for oil analysis from substations 1–3. A sample for estimate of abundance was taken from a point at station 2 previously used for this purpose (Thomas 1977).

### OIL EXTRACTION AND ANALYSIS METHODS

Two to 20 g of wet sediment from the upper 10 cm of the core were wet weighed in a tared, rinsed 50-mL glass centrifuge tube, and slurried with pentane-extracted distilled water (5 mL). The slurry was then extracted twice with 10 mL redistilled pentane for 20 min on a Burrell Wrist-action shaker at 800 oscillations/min.

The sediment–pentane mixture was centrifuged 10 min to separate the phases. The pentane supernatant was drawn off and the extraction repeated. The pentane extracts were pooled, concentrated to ca 5 mL (N-Evap), and transferred to a graduated centrifuge tube. Sufficient pentane was added to yield a final volume of 10.0 mL hydrocarbon extract in pentane. The extracted sediment was dried overnight in a drying oven at 110°C, and the dry weight recorded.

Hydrocarbon concentration in the pentane extract was determined fluorometrically ($\lambda_{exc}$ 310 m$\mu$, $\lambda_{em}$ 374 m$\mu$, Perkin-Elmer MPF-2A fluorescence spectrophotometer). Final concentrations were expressed as micrograms *Arrow* Bunker C oil "equivalents" per gram dry weight of sediment.

### TISSUES

All organisms were first thoroughly cleaned and rinsed under running tapwater to remove all traces of organic

TABLE 2. Species occurring in at least ¾ of all stations of each shore type and their mean upper and lower limits (metres above mean low water). ST, extending subtidally.

| Species | Upper limit | Lower limit |
|---|---|---|
| *Rocky shores* | | |
| *Cladophora* sp. | 1.04 | ST |
| *Fucus vesiculosus* | 1.36 | ST |
| *F. serratus* | 0.00 | ST |
| *Ascophyllum nodosum* | 0.70 | ST |
| *Chondrus crispus* | 0.20 | ST |
| *Sertularia pumilla* | 0.41 | 0.19 |
| *Balanus balanoides* | 1.15 | 0.21 |
| *Gammarus oceanicus* | 0.76 | ST |
| *Acmaea testudinales* | 0.34 | ST |
| *Littorina saxatilis* | 1.35 | 0.08 |
| *L. obtusata* | 1.23 | ST |
| *L. littorea* | 1.20 | ST |
| *Thais lapillus* | 0.72 | ST |
| *Mytilus edulis* | 1.39 | |
| *Sedimentary shores* | | |
| *Spartina alterniflora* | 1.42 | 0.85 |
| *Cladophora* | 1.19 | ST |
| *L. saxatilis* | 1.66 | ST |
| *L. littorea* | 1.38 | ST |
| *Mytilus edulis* | 1.12 | ST |

detritus and sediment. They were then rinsed thoroughly with acetone. Bivalves and snails were removed from their shells and again rinsed thoroughly with acetone. After air-drying for 5–10 min the samples were minced into tared, prerinsed, glass test tubes, and the wet weight of tissue recorded.

The tissue sample was homogenized for 2 min in 25 mL redistilled methanol–benzene with a Polytron Ultrasonic blender. The homogenate was filtered through prerinsed filter paper, and the remaining tissue homogenate was reextracted with methanol–benzene. The final extracts were pooled in a flat-bottom boiling flask.

To the combined filtrates 20 mL of pentane-extracted distilled water and 2 N KOH in methanol were added to yield a final 0.5 N solution. This mixture was saponified for 2 h by refluxing. The saponified sample was put through a series of aqueous NaCl washes and pentane extractions to yield a final single pentane-benzene phase. This extract was dried overnight with anhydrous $Na_2SO_4$.

The sample was concentrated to ca 5 mL, transferred to a graduated centrifuge tube, and sufficient pentane added to yield a final volume of 10.0 mL tissue hydrocarbons in pentane.

Hydrocarbon concentration was determined fluorometrically ($\lambda_{exc}$ 310 m$\mu$, $\lambda_{em}$ 374 m$\mu$, Perkin-Elmer MPF-2A fluorescence spectrophotometer). Concentrations were expressed as micrograms *Arrow* Bunker C oil "equivalents" per gram wet weight of tissue.

All glassware used was rinsed with double distilled solvents. All solvents used were redistilled in glass. *Arrow* Bunker C, courtesy of Dr E. Levy, Bedford Institute of Oceanography, served as standard.

### STATISTICAL METHODS

Results from the analysis of the communities at all 37 substations were analyzed in a nested analysis of variance. The analysis was set up to compare oiled and control stations as follows: (1) each tidal level (substation) for control stations was compared to the same level at oiled stations, (2) each control station (substations pooled) was compared to each oiled station, (3) pooled control stations were compared to pooled oiled stations, (4) each species at control stations was compared to the same species at oiled stations. Probabilities were obtained from the $F$ values between two variances.

TABLE 3. Number of species and species diversity index from oiled and control stations.

| Station no. | Number of species (diversity index[a]) Oiled | Control |
|---|---|---|
| | *Rocky shores* | |
| 1 | 29 (0.56) ((0.30)) | 30 (0.26) ((0.76)) |
| 3 | 23 (0.34) | 33 (0.52) |
| | *Sedimentary shores* | |
| 2 | 13 (0.04) | 17 (0.10) |
| 4 | 9 (0.04) | 13 (0.05) |

[a]Shannon-Weiner diversity index (Pielou 1966) based on abundance as a measure of importance.

TABLE 4. Species unique to oiled and control sites on rocky and sedimentary shores.

| *Rocky shores* Oiled | Control |
|---|---|
| *Xanthoria parietina* | *Parmelia* sp. |
| *Verrucaria* sp. | *Elachista fucicola* |
| *Gammarus setosus* | *Punctaria latifolia* |
| *Strongylocentrotus droebachiensis* | *Petalonia palmata* |
| *Podoceropsis* sp. | *Polysiphonia lanosa* |
| Chironomidae | *P. urceolata* |
| | *Taelia felina* |
| | *Cyathura polita* |
| | *Gammarus angulosus* |
| | *Hydrobia minuta* |
| *Sedimentary shores* Oiled | Control |
| *Scoloplos fragilis* | *Juncus balticus* |
| *Lineus ruber* | *Fucus vesiculosus* |
| *Onchidoris aspersa* | *Haminoea solitaria* |
| | *Macoma baltica* |

## Results

### INTERTIDAL BIOTA

Seventy-one species of animals and plants were identified from the collections, the highest number from any one station being 33 species. Many of the common species showed characteristic intertidal zonation. This

TABLE 5. Summarized abundance and biomass data for Chedabucto Bay study stations.

| | Stn. 1 | | Stn. 2 | | Stn. 3 | | Stn. 4 | |
|---|---|---|---|---|---|---|---|---|
| | Oiled | Control | Oiled | Control | Oiled | Control | Oiled | Control |
| Fauna individuals/m² | 14776 | 1963 | 17 | 157 | 144 | 1136 | 8 | 103 |
| Fauna fresh biomass g/m² | 59 | 58 | 13 | 17 | 67 | 3 | 5 | 190 |
| Fauna decalcified dry biomass g/m² | 11.9 | 10.3 | 2.7 | 4.0 | 9.0 | 0.4 | 0.9 | 17.1 |
| Fauna ash-free biomass g/m² | 8.0 | 7.8 | 2.3 | 2.3 | 6.2 | 0.4 | 0.7 | 9.6 |
| Flora fresh biomass g/m² | 2684 | 6498 | 337 | 2578 | 710 | 4941 | 1862 | 3542 |
| Flora dry biomass g/m² | 615 | 1787 | 69 | 455 | 143 | 1089 | 315 | 600 |
| Flora ash-free dry biomass g/m² | 437 | 1453 | 44 | 359 | 109 | 852 | 276 | 443 |

information is summarized in Table 2. The data do not show any consistent differences in zonation of common species between oiled and control sites.

Table 3 shows the number of species occurring at each station together with the Shannon-Weiner diversity index (Pielou 1966) calculated using both biomass and abundance as a measure of importance. The number of species at control sites was always as high or higher than at oiled ones of the same substrate type.

When oiled and control sites were compared for species found at only one or the other, it was found that on rocky shores, 10 species were unique to controls and 6 to oiled; on sedimentary shores, 4 to controls and 3 to oiled. None of these is really a common species in the area. Data are summarized in Table 4.

Table 5 presents summarized abundance and biomass data for all stations. So that comparisons are valid, only substations that were common to pairs of stations were included. The statistical analysis of the complete raw data for the 12 most common species revealed that there was a highly significant difference in biotic importance between oiled and control stations ($P < 0.05$). This difference was mainly attributable to significant differences in the importance of a few common species between oiled and control stations. These differences are summarized in Table 6.

The most obvious large difference was that of the biomass of flora between oiled and control. At oiled stations the mean fresh weight biomass was 1398 g/m² whereas at controls it was 4390 g/m² ($P$ for difference <0.005.). The analysis did not show any particular stations or levels to differ significantly from each other in producing the observed effects.

TABLE 6. Summary of significant differences in importance of species at oiled and control stations.

| Species | $P$ | Difference |
|---|---|---|
| *A. nodosum* | <0.005 | Biomass higher at control |
| *M. edulis* | <0.005 | Numbers higher at oiled |
| *L. obtusata* | <0.01 | Numbers higher at control |
| *L. saxatilis* | <0.05 | Numbers higher at control |
| *L. vincta* | <0.05 | Numbers higher at oiled |
| *F. serratus* | <0.1 | Biomass higher at oiled |
| *G. oceanicus* | <0.1 | Numbers higher at oiled |
| *C. crispus* | <0.1 | Biomass higher at control |
| *F. vesiculosus* | <0.1 | Biomass higher at control |
| *T. lapillus* | <0.1 | Numbers higher at control |

### POPULATION STATISTICS FOR *Littorina littorea* AND *Mya arenaria*

The analysis of length and weight data for collections from oiled and control sites revealed several consistent and highly significant differences (Table 7). Statistical analyses confirmed that the overall differences in both length and weight between oiled and control sites were highly significant ($P < 0.001$) for both *L. littorea* and *M. arenaria*. Comparisons between identical levels at oiled and control station pairs revealed similarly significant differences.

Tests for differences between all possible oiled-control, substation pairs at station 1 for both length

TABLE 7. Summarized length and weight data for *Littorina littorea* and *Mya arenaria* collected from oiled and control substations.

| Station | Level (m) | Mean length (cm) | Mean wt (g) |
|---|---|---|---|
| | *Littorina littorea* | | |
| 1 oiled | 0.3 | 1.71 | 1.7 |
| 1 oiled | 0.65 | 1.57 | 1.5 |
| 3 oiled | 0.3 | 1.64 | 1.6 |
| 1 control | −0.3 | 1.30 | 0.8 |
| 1 control | 0.0 | 1.24 | 0.5 |
| 1 control | 0.3 | 1.38 | 1.1 |
| 3 control | 0.0 | 1.71 | 1.9 |
| Overall oiled | — | 1.64 | 1.6 |
| Overall control | — | 1.41 | 1.1 |
| | *Mya arenaria* | | |
| 2 oiled | 0.65 | 2.91 | 3.6 |
| 4 oiled | 0.65 | 2.54 | 2.7 |
| 2 control | 0.65 | 3.57 | 8.3 |
| 4 control | 0.3 | 3.15 | 6.2 |
| Overall oiled | — | 3.36 | 3.2 |
| Overall control | — | 2.73 | 7.3 |

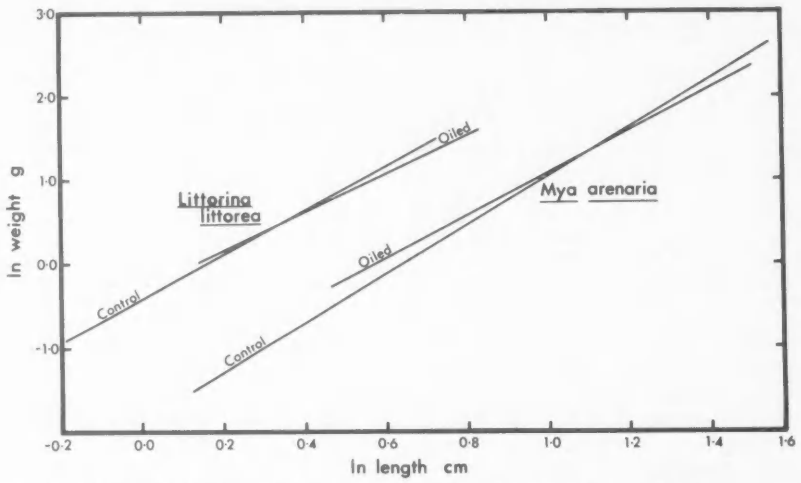


FIG. 2. Calculated length–weight regression lines for *Littorina littorea* and *Mya arenaria* from oiled and control stations.

and weight in *L. littorea* showed significant differences at better than the 99.9% level ($P < 0.001$) (12 cases). Tests between oiled substations showed no differences significant at this level (two cases). Tests between control substations showed only three of eight cases with differences significant at this level. At station 3 tests on length and weight of *L. littorea* (two cases) showed significant differences ($P < 0.001$) between oiled and control sites.

In the case of *M. arenaria* all oiled-control comparisons between substations, at each station, showed similarly significant differences ($P < 0.001$).

In summary, *L. littorea* at oiled stations were longer (mean 1.64 cm) and heavier (mean 1.6 g) than specimens from control stations (mean length 1.41 cm, weight 1.1 g). *Mya arenaria* at oiled stations were shorter (mean 2.72 cm) and lighter (mean 3.2 g) than specimens from control stations (mean length 3.36 cm, weight 7.3 g).

Calculation of the length–weight relationships for *L. littorea* and *M. arenaria* for control and experimental locations (pooled substation data) yielded the following equations (weight, grams; length, centimeters):

| | |
|---|---|
| *M. arenaria* | oiled ln wt. = 2.47 (ln length) −1.43 |
| *M. arenaria* | control ln wt. = 2.91 (ln length) −1.87 |
| *L. littorea* | oiled ln wt. = 2.27 (ln length) −0.30 |
| *L. littorea* | control ln wt. = 2.64 (ln length) −0.42 |

Differences between regression coefficients are significant at the 99% level ($P < 0.01$) for *M. arenaria* and at the 95% level ($P < 0.05$) for *L. littorea*. Regression lines are shown in Fig. 2. Both *L. littorea* and *M. arenaria* increase in weight more rapidly with length at control than at oiled stations.

Population density values for *L. littorea* and *M. arenaria* at the substations from which they were collected are shown in Table 8. These results show no significant differences between oiled and control stations.

Figure 3 shows length-frequency histograms for pooled samples of *L. littorea* and *M. arenaria* from oiled and control situations. These histograms do not show any notable differences between oiled and control situations other than different modes.

## OIL IN SEDIMENTS AND BIOTA

The quantities of oil extracted from sediment and biota samples from oiled and control stations are presented in Table 9. The sediment analyses confirmed that sediments at oiled stations were heavily contaminated with oil, whereas those at control stations showed only background levels. At oiled stations, oil concentrations were highest at mean high water, but still very high at mean tide level.

The table also presents results for two additional sediment samples. One was from station 2-oiled at a location where clam mortality has been monitored since 1970 and had persisted through 1976 (Thomas 1977). There, oil concentration was very high. A second additional sample was from Janvrin Harbour Lagoon which was only lightly oiled. There, oil concentrations were intermediate.

The oil content of the tissues of *L. littorea* from oiled locations averaged 12.18 μg/g whereas from controls it averaged 5.33 μg/g. These values are significantly different ($P < 0.01$). An additional sample from Arichat Church, an oiled location used in previous studies, was also higher than controls at 12.0 μg/g.

In the case of *M. arenaria*, the tissues of living specimens from oiled sites averaged 157.34 μg/g and from control sites, 60.73 μg/g. Individual values showed great variation and these differences were not significantly dif-

TABLE 8. Population density (no./m²) for *L. littorea* and *M. arenaria* from oiled and control substations.

| Station | Level (m) | No./m² |
|---|---|---|
| | *Littorina littorea* | |
| 1 oiled | 0.3 | 210 |
| 1 oiled | 0.65 | 339 |
| 3 oiled | 0.3 | 305 |
| 1 control | −0.3 | 860 |
| 1 control | 0.0 | 920 |
| 1 control | 0.3 | 470 |
| 3 control | 0.0 | 22 |
| Overall oiled | — | 284.7 |
| Overall control | — | 568.0 |
| | *Mya arenaria* | |
| 2 oiled | 0.65 | 4 |
| 4 oiled | 0.65 | 248 |
| 2 control | 0.65 | 16 |
| 4 control | 0.3 | 222 |
| Overall oiled | — | 126 |
| Overall control | — | 119 |

ferent. However, Gilfillan and Vandermeulen (1978) obtained much higher values from clams from the same oiled locations and the additional samples given here from newly dead clam tissues averaged 650.8 μg/g which is significantly higher than control levels ($P < 0.05$).

With *S. alterniflora*, samples from oiled locations showed a mean of 15 134 μg/g oil in their tissues while control grass showed a mean of 28.2 μg/g, the concentrations being highly significantly different ($P < 0.005$).

The amount of surface oil persisting on the surface at various tidal levels was generally low at oiled locations and always zero at controls. At station 1 no surface oil was present below the 1-m level and less than 1% of the shore between there and high water was contaminated. The situation at station 3 was similar except that the zero oil level was 0.65 m. At station 2 there was no surface oil below 1.0 m, less than 1% from 1.0 to 1.3 m, and 30% from 1.3 to 1.9 m. At station 4 there was no surface oil below 1.1 m, 5% from 1.1 to 1.6 m and less than 1% from 1.6 to 1.91 m.

A sample of *M. arenaria* from station 2-oiled for comparison with previous samples from this location (Thomas 1977) showed an abundance of 560/m².

## Discussion

Ecologists attempting to determine the affects of oil spills in poorly studied locations face particular problems. Data on normal intertidal communities is usually fragmentary or absent and access to the coastline is often difficult. These problems occurred in Chedabucto Bay and were compounded by the fact that the spill occurred in the coldest part of winter when sea-ice formation was considerable and, consequently, many shores were impossible to observe or sample. It was even difficult to determine the exact extent of oil contamination because of movement in and under ice (Anon. 1970). At the time, it was impossible to make precise, detailed studies of newly oiled situations. Consequently, it was the spring of 1970 before areas were examined in detail and by then biotic effects had already clearly occurred (Thomas 1973).

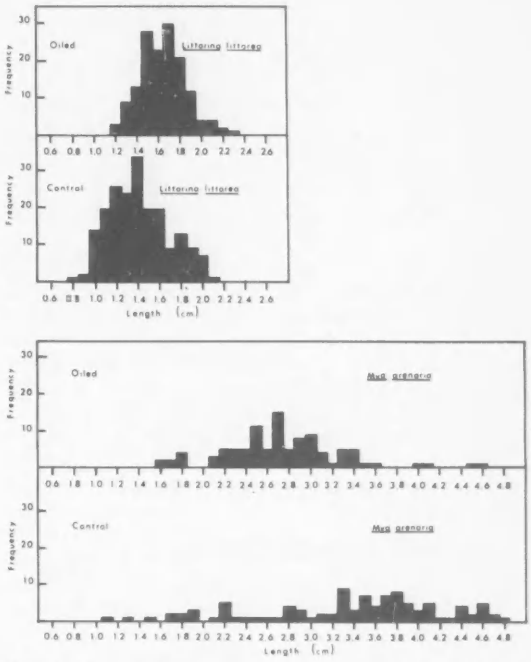


FIG. 3. Length-frequency histograms for *L. littorea* and *M. arenaria* from oiled and control stations.

The difficulty of providing an adequate control situation was aggravated by these conditions and it was decided that a double approach would be used. Detailed surveys of six sample sites would be repeated as often as practicable on the assumption that with time, oil effects would change and the biota would respond. It was assumed that eventually intertidal communities would return to a normal condition and therefore provide a self-control. A second control was attempted in the comparison of oiled sites with a reasonably close-by unoiled control. Unfortunately, however, this location was contaminated with remobilized oil and also suffered extensive natural storm damage.

The method of using time as a control was shown to be useful but lacking in sensitivity (Thomas 1977). Only large changes in common organisms could be correlated with oil pollution. It must also be realized in using this method that climatic changes may cause progressive biotic changes. Such cases have been described by Crapp (1971), Baker (1976), and Straughan et al. (1978). Nevertheless, it was possible to attribute large

TABLE 9. Quantities of oil extracted from sediments and biota from Chedabucto Bay subsamples. (Sediments, micrograms per gram dry weight; biota, micrograms per gram wet weight.)

| Station | Level (m) | Oil extracted (duplicates) μg/g ABC equivalents | |
|---|---|---|---|
| | *Sediments* | | |
| 2 oiled | 0.65 | 254.26 | 90.27 |
| 2 oiled | 0.70 | 11.52 | 0.81 |
| 2 oiled | 1.00 | 11 020.22 | 9 923.81 |
| 2 oiled | 1.30 | 19 926.33 | 16 129.79 |
| 2 control | 0.30 | 0.31 | 0.32 |
| 2 control | 0.65 | 1.98 | 3.71 |
| 2 control | 1.00 | 5.54 | 5.20 |
| 2 control | 1.30 | 0.88 | 0.24 |
| 2 control | 1.91 | 0.81 | 0.59 |
| 4 oiled | 0.65 | 2 644.92 | 74.42 |
| 4 oiled | 1.00 | 4 952.92 | 9 493.66 |
| 4 oiled | 1.30 | 18 433.48 | 25 577.74 |
| 4 control | 0.3 | 0.35 | 0.21 |
| 4 control | 0.65 | 0.26 | 0.24 |
| 4 control | 1.00 | 0.00 | 0.00 |
| | *Additional sediment samples* | | |
| 2 oiled SS no. 2 Janvrin Hbr. | | 11 747.77 | 10 344.47 |
| Lagoon | 0.65 | 168.62 | 185.19 |
| | *Littorina littorea* | | |
| 1 oiled | 0.30 | 11.0 | 11.3 |
| 1 oiled | 0.65 | 18.2 | 19.0 |
| 1 control | −0.30 | 6.9 | 10.2 |
| 1 control | 0.00 | 4.6 | 5.8 |
| 1 control | 0.30 | 6.0 | 4.0 |
| 3 oiled | 0.30 | 5.9 | 7.7 |
| 3 control | 0.00 | 2.5 | 2.5 |
| | *Additional L. littorea samples* | | |
| Arichat Church — oiled | 0.00 | 9.2 | 16.6 |
| | *Mya arenaria* | | |
| 2 oiled | 0.65 | 52.4 | 48.8 |
| 2 oiled | 0.70 | 51.0 | 34.7 |
| 2 control | 0.65 | 47.5 | 143.3 |
| 4 oiled | 0.65 | 167.2 | 110.7 |
| 4 control | 0.30 | 29.2 | 22.9 |
| | *Additional M. arenaria samples* | | |
| 2 oiled | SS no. 1 alive | 56.1 | 73.3 |
| 2 oiled | SS no. 2 alive | 183.3 | 981.0 |
| 2 oiled | SS no. 2 newly dead | 946.5 | 882.3 |
| 2 oiled | SS no. 3 alive | 59.1 | 69.9 |
| 2 oiled | SS no. 3 newly dead | 123.6 | — |
| | *Spartina alterniflora* | | |
| 2 oiled | 1.3 | 10 351.8 | 9 028.7 |
| 2 control | 1.6 | 67.8 | 15.0 |
| 4 oiled | 1.0 | 20 943.5 | 20 215.4 |
| 4 control | 0.65 | 15.4 | 14.7 |

changes in common and important species in Chedabucto Bay to oil pollution. The dominant fucoid algae of rocky shores were reduced in vertical distribution, salt marsh grasses suffered delayed mortalities, and soft-shell clams in lagoonal sediments declined in numbers. It was also shown that mortalities may be delayed up to several years from initial oiling as was the case with salt marsh cord grass. It was also demonstrated that common fauna such as periwinkles and barnacles were not obviously affected and that in the case of barnacles, reproduction was consistent even while contamination was heavy.

The detailed comparison of four oiled and four control stations described here has yielded further useful information and was also used by Straughan (1977) to identify effects of the *Metula* crude oil spill in the Straits of Magellan. This method, however, also lacks sensitivity. The basic problem is the large fundamental variability of shallow water marine and intertidal communities. Even in a single location, such associations show high variability only a part of which can be correlated with known physical variables (Hughes and Thomas 1971a, b). When control and experimental sites must be geographically separated the unaccountable variance becomes even higher. This tends to mask the more subtle but nevertheless important effects.

Both the time and space control techniques for delimiting the long-term effects of oil on intertidal communities are valid and useful. The information obtained is useful in predicting the effects of spills and cleanup procedures in diverse habitats. When combined with experimental data it suggests profitable future research projects. However, only comparatively large changes are documented by these methods and such changes are likely to have more widespread ecological effects than can be readily observed.

This study has shown several additional biotic effects of oil in Chedabucto Bay, and confirmed previous ones. No significant differences in intertidal distribution of species between oil and control sites were observed. Thomas (1977) had previously observed that such differences had disappeared by 1976. There was, however, a very consistent difference in the species diversity, as shown by the number of species found, between oiled and control sites. This situation is typical of communities stressed by pollution but it is interesting that the difference persists over 6 yr after the disaster, especially since most shores are essentially oil free. Diversity indices did not prove useful because results varied widely according to the measure of importance used to calculate them. Biomass is the most useful comparative measure of species importance in intertidal areas since many species are compound, colonial, or encrusting and difficult to count. However, diversity indices based on biomass give low and misleading values. In the case of station 1, the use of biomass and numbers as measures of importance gave opposite results (Table 3). Another aspect of diversity differences was shown by the larger number of species unique to control than oiled stations on rocky and sedimentary shores. While some of these occurrences are undoubtedly by chance, others may be

sensitive species. Of particular interest may be *Polysiphonia lanosa*, a red alga epiphytic/parasitic on *Ascophyllum nodosum*, which is normally abundant, but was not found in oiled station samples.

Computer analysis of the complex matrix of data generated by the experiment yielded relatively few significant differences. The overall difference in importance value between oiled and control situations was highly significantly different but no one level, group of levels, or stations showed significant differences from others. The data show that the biomass of flora (algae and flowering plants) was very significantly lower at oiled sites. This is consistent with large effects on flora described previously by Thomas (1977). However, this new data shows a more widespread effect. Three algae had significantly higher biomass levels at control than oiled situations. These were *A. nodosum*, the knotted wrack, *Chondrus crispus*, Irish moss, and *F. vesiculosus*, bladder wrack. The first two are common and of commercial importance; the latter is often dominant on exposed rocky shores. Only one alga, *F. serratus*, which occurs in the lower shore was significantly more important at oiled than control stations. All the fucoid algae species present in the area have now been shown to be affected by oil pollution.

Among the fauna the differences were generally less noticeable. Three species of gastropods showed significantly higher numbers at control sites. These were two periwinkles, *L. obtusata* and *L. saxatilis*, and the dog whelk *T. lapillus*. Showing the opposite trend were the bivalve *M. edulis*, the blue mussel, the gastropod *L. vincta*, the chink shell, and the amphipod crustacean *Gammarus oceanicus*. It is interesting that the two periwinkles were less abundant at oiled sites, because the third common member of this genus, the common periwinkle *L. littorea*, which was equally abundant throughout, did show significant length, weight, and length–weight ratio differences between oiled and control sites.

The two most common animals of rocky and sedimentary shores, *L. littorea* and *M. arenaria*, were selected for size studies. Neither showed significant differences in abundance between oiled and control sites but differences in length and weight were highly significant. *Littorina littorea*, the common periwinkle, was longer and heavier at oiled sites, whereas the soft-shell clam *M. arenaria* showed the opposite. Both, however, showed a slower increase in weight per unit of length at oiled than at control situations. It is known that oil affects both species. *Littorina littorea* shows increased crawling speed and metabolic rate with low doses of Bunker C oil (Hargrave and Newcombe 1973) and narcotization at higher levels (Griffith 1972) but generally not mortality. *Mya arenaria* may show increased or decreased metabolic rates and feeding rates in varying degrees of oil production (Anderson 1972; Avolizi and Nuwayhid 1974; Stainken 1978). Heavy pollution often results in significant mortalities (Michael 1977; Thomas 1977), and population declines associated with oil pollution have been demonstrated in Chedabucto Bay and elsewhere. It appears that oil pollution also restricts growth in this species (Gilfillan and Vandermeulen 1978), and alters the length–weight ratio. Growth in weight is apparently reduced more than growth in length. Length frequency studies show that the smaller mean size in oiled areas is not caused by large numbers of small clams. The periwinkles showed a similar affect in the length–weight relationship but individuals in oiled locations were significantly longer and heavier than at control sites. Again length–frequency determinations suggested a growth rate effect rather than a population structure effect. The reason for the difference could be associated with the algal changes observed. *Littorina littorea* browses on rock surfaces and reduction in algal biomass may well result in increased growth of microflora that was formerly shaded. It is noteworthy that recruitment to populations of species adversely affected by oil has not been affected. Settlement and growth have been observed in *Fucus* sp., *M. arenaria*, and *Balanus balanoides* annually since the spill (Thomas 1977). Apparently in affected species the postsettlement period is most vulnerable with observed effects on both growth and survival.

Oil analyses of sediments and biota were useful in confirming the general information regarding the oil pollution situation at oiled and control sites. Sediments from oiled locations proved to still contain very high concentrations of oil, the highest being 2.5% at high tide level at lagoonal station 4. Oil concentrations at midtide levels in lagoons were generally an order of magnitude lower, but still high. In these locations visible surface oil remains only at high tide level.

Oil concentrations in animals were significantly higher at oiled than control situations but not strikingly so. Other data for the same general areas show much higher levels in biota (Gilfillan and Vandermeulen 1978). It was noteworthy that the oil concentration in living clams from a substation at station 2 where mortality continues to be above normal (Thomas 1977) were very high, and levels from newly dead clam tissues from this location were even higher. There is no doubt that clam mortalities are associated with oil contaminated sediments and that oil concentrations are higher in dying and dead clams than in others.

Bunker C oil is clearly implicated in the mortalities of several dominant and important intertidal species in Chedabucto Bay. Of particular concern are reductions in abundance, biomass, and distributions of several major primary producers. Such changes alter the energy flow patterns in the communities and inevitably have broad ecological effects. On the shores of southern England, where algae and animals suffered heavy mortalities following the cleanup of the *Torrey Canyon* spill, Southward and Southward (1978) have documented large unbalanced changes in the biota, which may take a long time to stabilize. Mortalities in Chedabucto Bay were not nearly so extensive as in England but did affect

all shores studied. The great increase in common periwinkle size on oiled shores is perhaps a good example of a consequent trophic change; others are no doubt occurring but difficult to document. Further study may give useful information.

Studies in Chedabucto Bay allow us to predict major biotic changes that would occur following a similar spill. There is still a problem, however, in making recommendations concerning cleanup. On rocky shores evidence suggests that cleanup measures would not hasten recovery and might even delay it, since their biotic effects would be as harmful as those of the oil. In sheltered sedimentary locations, however, oil is much more persistent and becomes incorporated deep into sediments where degradation is slow. If cleanup methods for lagoons could be improved so that oil could be removed without sediment penetration or disturbance, cleanup should help to minimize oil pollution effects.

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# *Florida* Oil Spill Impact on the Buzzards Bay Benthic Fauna: West Falmouth[1,2]

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SANDERS, H. L. 1978. *Florida* oil spill impact on the Buzzards Bay benthic fauna: West Falmouth. J. Fish. Res. Board Can. 35: 717–730.

No matter what criterion is used to measure the effects of the *Florida* oil spill, the densities and species composition and the array of statistical methods demonstrate that the same hierarchical pattern emerges. Densities and species composition remain stable over time at the minimally oiled and unoiled stations, but display considerable fluctuations and marked changes at the more heavily oiled stations. With simple presence or absence data, highest fidelity is present at the marginally oiled stations, lower fidelity at the intermediately oiled stations, and lowest fidelity at the severely oiled stations. The discrepancy index measures mean yearly differences in faunal composition at each of the stations. Very large and large differences are documented for the severely and intermediately oiled stations but only small differences are found for the marginally oiled stations. The coefficient of variation is a measure of faunal variability throughout the entire sampling period for each of the stations. Faunal variation remains very high at the severely and intermediately oiled stations but low at the marginally oiled sites. Cluster analysis reveals profound temporal changes in the fauna from samples collected at the severely and intermediately oiled stations but demonstrates a much more homogeneous pattern with only small seasonal changes from samples obtained at the marginally oiled stations.

*Key words:* benthos, hydrocarbon, intertidal zone, long-term effects, petroleum, pollution, statistics, subtidal zone

SANDERS, H. L. 1978. *Florida* oil spill impact on the Buzzards Bay benthic fauna: West Falmouth. J. Fish. Res. Board Can. 35: 717–730.

Quel que soit le critère utilisé pour mesurer les effets du déversement accidentel de pétrole du *Florida*, la densité du benthos et sa composition par espèces, ainsi que toute une série de méthodes statistiques aboutissent à un même schéma hiérarchique. Les densités et la composition par espèces demeurent stables aux stations peu ou pas touchées par le pétrole, mais accusent de vastes fluctuations et des changements marqués aux stations où le pétrole est plus abondant. Utilisant de simples données de présence ou d'absence, on observe la plus grande uniformité aux stations marginalement touchées par le pétrole, une uniformité moindre aux stations intermédiaires et l'uniformité la plus faible aux stations fortement touchées par le pétrole. L'indice de différence sert à mesurer les différences annuelles dans la composition faunique à chacune des stations. On constate de très grandes et de grandes différences aux stations fortement touchées par le pétrole et aux stations intermédiaires, mais seulement de petites différences aux stations marginalement touchées. Le coefficient de variation est une mesure de la variabilité faunique au cours de l'entière période d'échantillonnage à chacune des stations. La variation faunique demeure élevée aux stations fortement touchées par le pétrole et aux stations intermédiaires, mais est faible aux stations marginalement touchées. L'analyse par groupements numériques révèle de profonds changements temporels dans la faune des échantillons prélevés aux stations fortement touchées par le pétrole et aux stations intermédiaires, mais démontre une configuration beaucoup plus homogène, avec petits changements saisonniers seulement, dans les échantillons prélevés aux stations marginalement touchées.

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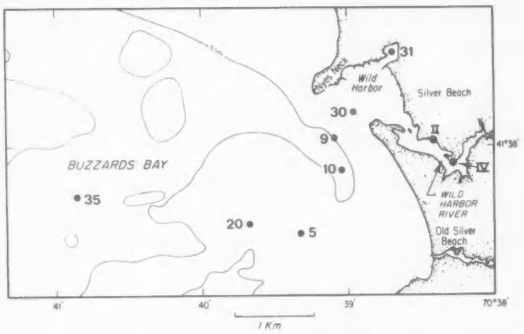


FIG. 1. Location of the intensively studied stations in Wild Harbor River, Wild Harbor, and Buzzards Bay. The Sippewissett Marsh station is not shown.

IN the early hours of September 16, 1969, the barge *Florida* ran aground and rent its hull on Fassett's Point off West Falmouth, Mass. About 650 000–700 000 L of No. 2 fuel oil escaped into the waters of Buzzards Bay. Strong south–southwest winds moved much of the oil into the Wild Harbor and Wild Harbor River area of North Falmouth. The shoreline of Wild Harbor and Wild Harbor River soon became littered with continuous rows of dead and dying fish and marine invertebrates. From the composition of the dead and moribund animals it was obvious that a massive kill was occurring in the intertidal and immediate subtidal areas of Wild Harbor.

Because of the close proximity of the Woods Hole Oceanographic Institution to the site of the accident, scientists from that organization were able to exploit this unique opportunity by undertaking and completing a study of the biological effects of this oil spill with sufficient rigor and detail and of long enough duration so as to yield a body of sound and solid data upon which to base a set of valid conclusions. Such critical requisites are clearly not the hallmark of most benthic community studies concerned with oil pollution. These shortcomings, in my opinion, are one of the major reasons for the deplorable state of our knowledge of the effects of oil on marine life. Because the West Falmouth oil spill proved to be so extensive (H. L. Sanders et al. unpublished data), I will be able to convey only the briefest summary of its content. I will not attempt to include the many themes considered but instead will limit my comments to the responses over time of a series of stations taken along an oil-induced stress gradient.

A series of subtidal and intertidal stations was established that ran from Wild Harbor and Wild Harbor River, where the maximum impact on the marine biota was evident, into the main body of Buzzards Bay. Figure 1 gives the locations of all our intensively studied stations except the intertidal control station in Sippewissett Marsh (2.9 km south of Wild Harbor River) which remained uncontaminated by the No. 2 fuel oil and contained the same suite of intertidal species present in the Wild Harbor River. Our long-term research strategy was to reoccupy certain stations on the basis of differing severities of contamination from very heavily oiled inshore to very lightly oiled offshore sites. By sampling each station at short time intervals changes in oil concentration and composition were carefully monitored. The resultant petroleum hydrocarbon data were then related to the detailed analyses of the concomitant biological samples.

Three distinct responses became evident from the oiling of the sediments of Wild Harbor, Wild Harbor River, and the adjacent seafloor of Buzzards Bay which can be separated on the following criteria: (1) severely oiled sites, containing from 40 to 450 ppm of hydrocarbons, as exemplified by intertidal stations II and IV and subtidal station 31; (2) intermediately oiled sites, with 15 to 30 ppm of hydrocarbons, represented by subtidal stations 9, 10, and 30; (3) marginally oiled and unoiled sites, 2.5–8 ppm hydrocarbons, present at subtidal stations 5, 20, and 35 and the intertidal Sippewissett Marsh station.

## Materials and Methods

Biological samples were collected with a 1/127 $m^2$ corer at the intertidal stations and with a 1/25 $m^2$ van Veen grab at the subtidal stations. The samples were washed through a 0.297-mm standard mesh screen, and the component retained was preserved in 5% formalin, transferred to 70% alcohol after 24 h, and stained in Rose Bengal to aid sorting. All animals were separated from the sediment and detritus under a microscope and sorted by species. A small aliquot of sediment obtained from each biological sample was frozen for oil analysis and another small quantity of sediment was retained for particle-size analysis. The intensively studied stations were sampled at monthly or bimonthly intervals. The raw data were analyzed by a number of statistical methods. The examples shown have been taken from the extensive and detailed report on the West Falmouth oil spill documenting the responses over time of the stations along the oil-induced stress gradient (H. L. Sanders et al. unpublished data).

### FIDELITY

This simple statistic is a measure of whether or not a species is a consistent or infrequent faunal constituent of a given station and it is determined on a presence or absence basis. If a species is present in a high percentage of the samples, then fidelity is high. Conversely, if the species occurs in only a small percentage of the samples, then its fidelity is low. The theoretical upper limit of fidelity is achieved when each species is present in every sample (1.00). Conversely, the theoretical lower limit of fidelity is realized when each species is present in only one of a series of samples (1/number of samples).

### COEFFICIENT OF VARIATION

This statistic is used to measure the stability or evenness of an individual species or the total fauna at a given station over time. It is determined merely by dividing the standard

deviation by the mean density. A low coefficient of variation means that numerical densities for that species remain relatively constant over the time period considered, i.e. the species is in equilibrium. Alternatively, a high coefficient of variation denotes considerable numerical variation or instability for the species over the same time interval, i.e. a nonequilibrium condition persists.

### Discrepancy Index

We have developed a statistical formulation to measure yearly differences called the discrepancy index, whose derivation is as follows:

Let

$$Z_n = \frac{\max\,(x_t, y_t)}{\min\,(x_t, y_t)} \cdot (x_t + y_t),$$

where $x_t$ is the average number of individuals for one year and $y_t$ is the average number of individuals for the following year per 1/25 $m^2$ for the subtidal stations and per 1/127 $m^2$ for intertidal station IV.

$Z_n$ is ranked by $Z$ order as follows:

$$Z_1 \geq Z_2 \geq Z_3 \ldots \geq Z_n$$

so that $x_t$ and $y_t$ become $x_n$ and $y_n$.

The set of species, $C_n$, is defined

$$C_n = \{K \mid Z_k \leq Z_n\}.$$

The discrepancy index is defined

$$d_n = \frac{\sum_{k \in C_n} Z_k}{\sum_{k \in C_n} (x_k + y_k)}.$$

The contribution that each species makes to the discrepancy index is determined by two conditions: (1) sum of each species' two mean densities for the 2 yr studied (the horizontal vector in Fig. 7) and (2) the ratio of its larger average density to its smaller average density for the 2 compared yr (the vertical vector in Fig. 7). However, when using this definition in cases where species are present one year but not the other, the resulting ratios take on the value of infinity. To circumvent this difficulty, a single individual is assigned to that species for the year it was missing. Therefore, the mean density for that year becomes 1/number of samples rather than 0.

If the species composition is identical for the 2 yr that are compared and the mean densities of each species for the 2 yr are identical, then the discrepancy index is graphed as a straight line with a value of 1.0. Such total coincidences yield the minimum possible discrepancy index and serve as our baseline. Progressive departures from this baseline, as represented by ever larger discrepancy values, become measures of increasing faunal differences between the 2 yr.

### Cluster Analysis

This methodology is used to determine faunal similarity among an array of stations or samples. Employing this procedure, the most faunally similar samples are grouped together first. These, in turn, join to form the next most faunally similar larger groupings. At progressively smaller similarity values, these larger groupings combine into increasingly larger clusters until the least similar aggregations, the primary clusters, are achieved. The cluster analysis variant NESS (the normalized expected species shared) was used in the West Falmouth study and only samples having 100 or more individuals are included in the analyses (see Grassle and Smith 1976). Some similarity measurements are primarily determined by the dominant species in the samples, while others are based on mere presence or absence and not on relative abundance. NESS is a less biased technique in that it weights more validly the contributions of rare and common species to this determination.

NESS similarity depends on taking random picks of a preset number of individuals within a population or sample and comparing that sample with the same preset number of individuals drawn from another population or sample and then determining the number of species shared. In the present case, the preset number of individuals is 50. When the random picks are repeated a number of times, the average number of species shared between the paired random samples from the two populations will, by the law of large numbers, approach a fixed value, the expected species shared. The expected species shared forms the basis of the similarity measure.

## Results and Discussion

### Densities and Species Composition

The most obvious feature of the samples collected in this study was their overwhelming numerical dominance by two capitellid polychaete species, *Capitella capitata* in the 1st yr at intertidal stations II and IV and subtidal station 31 and *Mediomastus ambiseta* at the other subtidal stations during the 2nd and 3rd yr of study. *Capitella* contributed from 62.5 to 99.9% of the specimens present in 19 of the 25 samples obtained at stations II, IV, and 31 from September 1969 through August 1970. *Mediomastus* numerically made up from 51.5 to 93.9% of the individuals in 26 of the 35 samples collected after July 1970 at stations 5, 9, 10, 20, and 35.

In response to the nearly total decimation of the benthic fauna in the area of maximum oil pollution immediately following the oil spill (exemplified by intertidal stations II and IV and subtidal station 31), there was an explosive increase in the opportunistic polychaete *Capitella capitata*. With an exponential numerical increase to densities of tens of thousands to more than 200 000 individuals/$m^2$ composed almost entirely of *Capitella* and the persistence of these highly elevated densities through June 1970, the intertidal flats of the Wild Harbor River and the neighboring subtidal bottoms were converted from December 1969 to July 1970 into a remarkedly dense unispecies culture of *Capitella*. There was a rapid decrease in *Capitella* density during July, August, and September 1970. Thereafter, there were sudden, abrupt, and marked changes in densities, species numbers, and species composition from one sampling date to the next, particularly at station 31.

The intertidal Sippewissett control samples revealed considerably more stability regarding total density, species numbers, and faunal composition than did the samples collected from the Wild Harbor River stations

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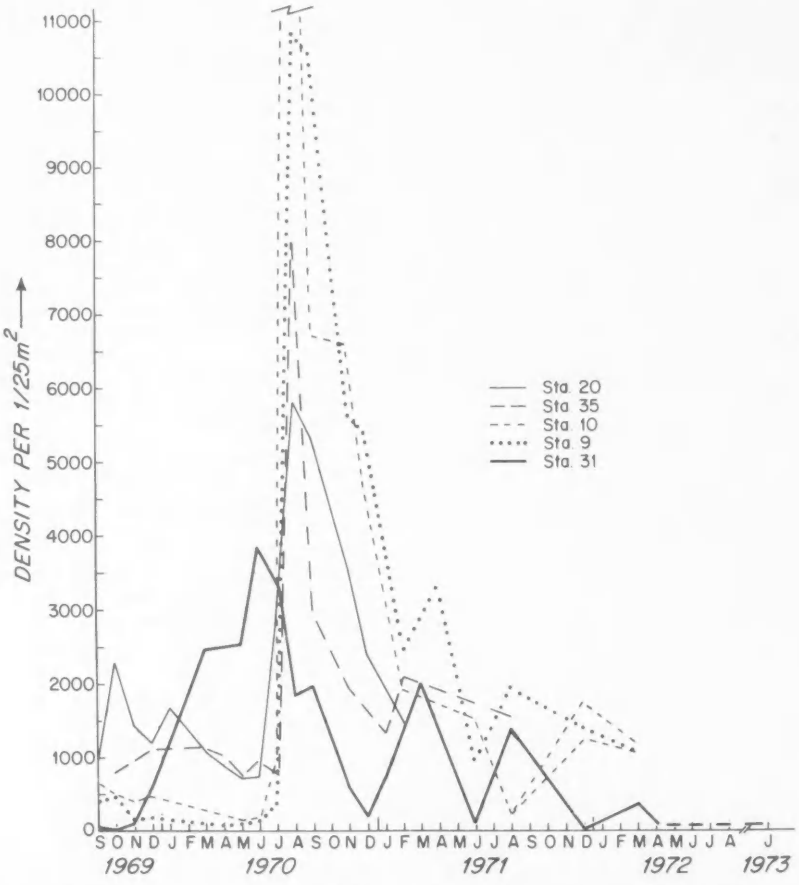


FIG. 2. Animal densities per 1/25 m² obtained at stations 31, 9, 10, 35, and 20.

II and IV. These findings support the interpretation that the differing temporal patterns evident at stations II and IV and the Sippewissett stations were a response to the oil-induced stress in the Wild Harbor estuary.

The pattern in the intermediately oiled sediments from the outer reaches of Wild Harbor in Buzzards Bay as documented by stations 9 and 10 showed a marked depression in animal numbers and species in the aftermath of the oil spill from September 1969 through July 1970 (Fig. 2 and 3). During this period of time, the habitat was "biologically undersaturated." Within the brief interlude of a month, from July to August 1970, the benthic density increased 30 and 23 times at stations 9 and 10, respectively, while the species numbers rose from 18 to 61 at station 9, and 34 to 83 at station 10. The fauna was composed almost totally of very young individuals that had recently settled onto the bottom from the overlying water. This phenomenon can be related to the fact that the majority of benthic species in this geographic region are summer breeders. These very high densities were immediately followed by a precipitous drop in numbers during the fall, winter, and spring of 1970–71 to the low density levels found in September and October 1969. Such extremely high mortality rates for the benthic invertebrates in the 2nd yr after the oil spill revealed that the benthic fauna had not recovered in this time period. The higher species numbers coupled with intermediate densities and less variation in the 3rd yr, particularly at station 10, were indicative of some recovery.

The faunal density patterns at the minimally oiled stations 5, 20, and 35, in similar depths to those present at stations 9 and 10, differed considerably from the patterns described for the latter stations. Excluding

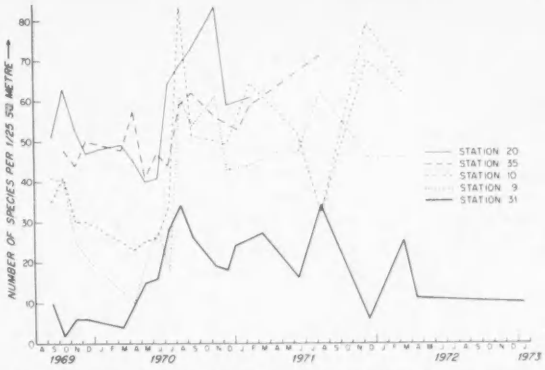


FIG. 3. Number of species per 1/25 m² obtained at stations 31, 9, 10, 35, and 20.

*Mediomastus* (Fig. 4), faunal densities averaged nearly an order of magnitude higher at stations 5 and 20 from September 1969 through July 1970 than did the densities at stations 9 and 10. There was no obvious increase in faunal numbers in the August 1970 samples at stations 5, 20, and 35 when densities increased 30- and 23-fold at stations 9 and 10. Unlike the precipitous drop in density found at stations 9 and 10 from August 1970 to at least the end of the year, densities at stations 5, 20, and 35 remained relatively constant during the same time interval.

## FIDELITY

The results, presented in Fig. 5, include species found in more than 45% of the samples at stations 31, 9, 10, 5, 20, and 35. These species are ranked by station from highest to lowest fidelity.

Three sharply different patterns emerge. The 20 samples from severely oiled station 31 yielded a mere 6 species in the 0.45–1.00 fidelity range. No species was present in all of the station 31 samples and the maximum fidelity was 0.90. Twenty-nine and 34 species at intermediately oiled stations 9 and 10 had fidelity values

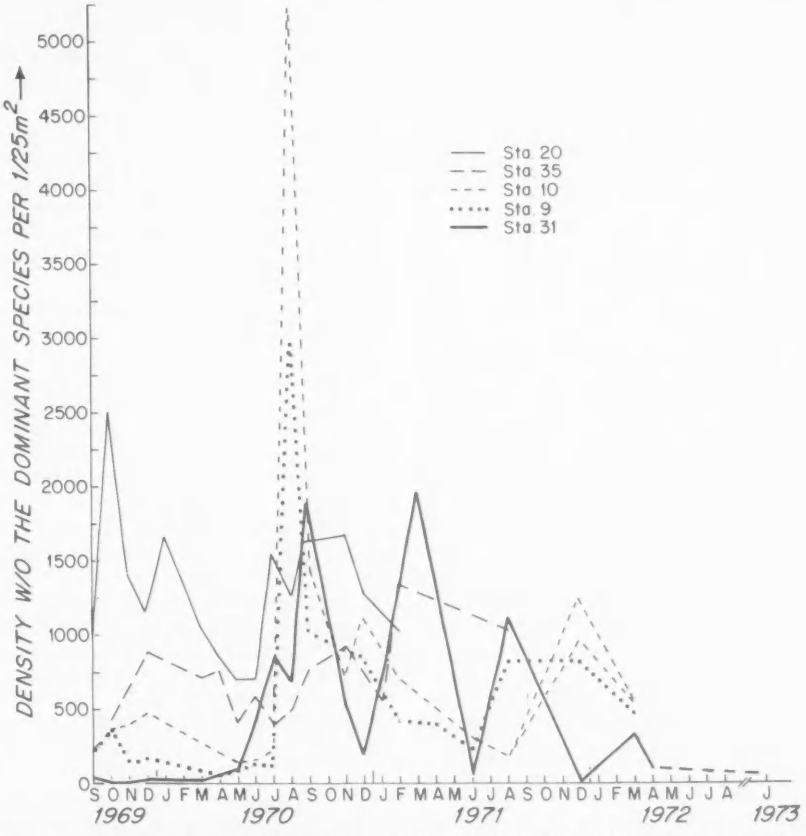


FIG. 4. Densities per 1/25 m² obtained at stations 31, 9, 10, 35, and 20 with the exclusion of the numerically dominant species *Mediomastus ambiseta*.

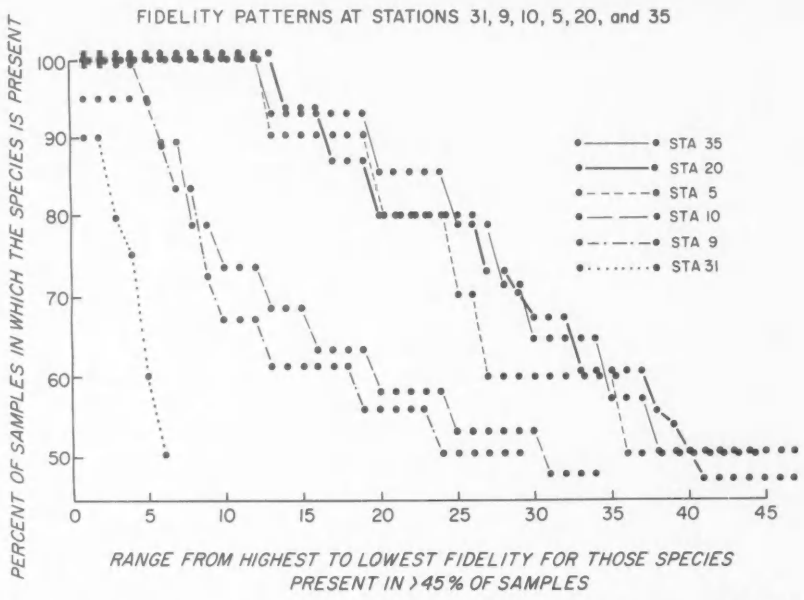


FIG. 5. Fidelity patterns at stations 31, 9, 10, 5, 20, and 35. Species that were present in more than 45% of the samples at each of stations 31, 9, 10, 5, 20, and 35 are ranked along the abscissa from highest to lowest fidelity.

in the 0.45–1.00 range. Four species achieved fidelity values of 1.00 at station 10. The total number of species per station with fidelity values >0.45 were more numerous at marginally oiled stations 5, 20, and 35 — 44 species at station 5 and 47 species at each of stations 20 and 35. There was also a marked increase in the number of species with fidelity values of 1.00 — 12 at stations 5 and 35 and 13 at station 20.

Thus, very few species were faunal components in half or more of the samples collected at severely oiled station 31. Such a pattern reflects the rapid faunal displacement and the abrupt, frequent, and pronounced density changes. The many high-fidelity species that characterize the minimally oiled stations 5, 20, and 35 are indicative of faunal stability that persisted at these stations throughout the study period. The smaller number of high-fidelity species at intermediately oiled stations 9 and 10 are products of the pronounced reduction in densities and species in the aftermath of the oil spill and the subsequent changes in faunal composition.

## COEFFICIENT OF VARIATION

The analysis is based on the intensively sampled stations 20, 35, 10, 9, and 31. A species must be present at a mean density of 3 or more individuals at a given station in order to be included in the analysis for that station. The number of species that qualify for stations 31, 9, 10, 20, and 35 are 14, 23, 36, 36, and 40, respectively. The species with the requisite mean densities of $\geq 3$ individuals are plotted by rank in order of increasing coefficients of variation at each of the five stations (Fig. 6). Thus the median or 50% coefficient of variation values for stations 31, 9, 10, 20, and 35 are the 7th, midvalue between the 11th and 12th, the 18th, the 18th and the 20th.

Largest coefficients of variation, except at the very highest ranks, are present at station 31. Next largest values, although well below the plot for station 31, are found at stations 9 and 10. The coefficients of variation, on the average, are much lower for species found at stations 20 and 35 (which are located at the minimally affected end of the oil-induced stress gradient) than at stations 10, 9, and particularly, 31, where the impact of the oil was significantly more severe. Note that the species occupying the median or 50% ranking of increasing coefficients of variation at stations 20 and 35 yield lower coefficients than all but the very lowest values present at stations 31, 9, and 10.

The null hypothesis stating that the coefficients of variation for the suite of species having mean densities $\geq 3.0$ at one station are not significantly different from the coefficients of variation for the suite of species having mean densities $\geq 3.0$ at another station in Fig. 5 was tested. The curves for stations 20 and 35 are not significantly different ($P = 0.472$) nor are the curves for stations 9 and 10 ($P = 0.478$). The curves for stations 20 and 35 do differ significantly from those for stations 10, 9, and 31 at $P < 0.01$. Station 10 and 31 curves differ significantly at a $P$ value of 0.067, the stations 9

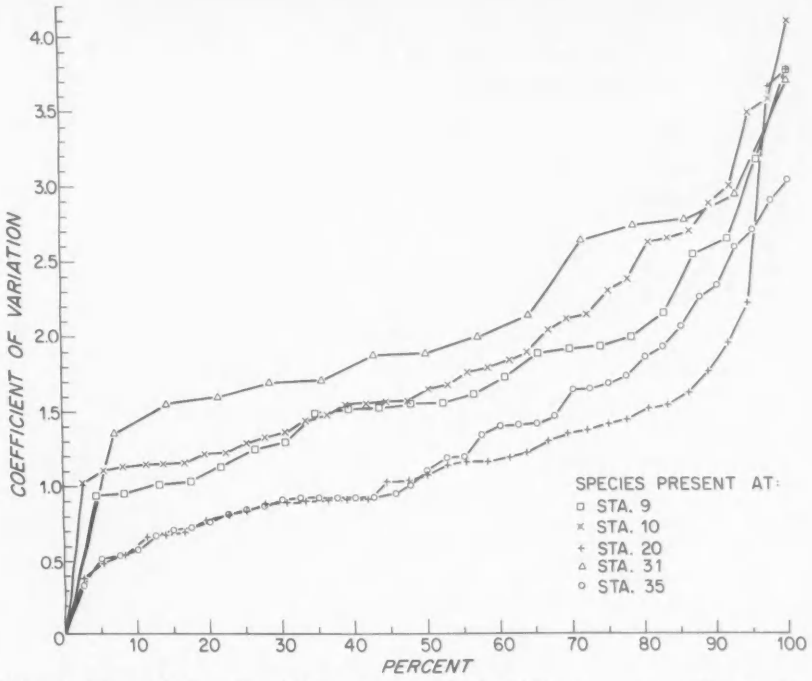


FIG. 6. The coefficient of variation values (standard deviation/mean) at stations 35, 31, 20, 10, and 9. For a species to qualify, it must be present at a mean density $\geq 3.0$ individuals at one or more of the five stations. Species are plotted in order of increasing coefficients of variation at each station.

and 31 curves are also significantly different at a $P$ value of 0.052. These findings clearly support the contention that the coefficient of variation patterns for these stations are the products of the oil-induced stress gradient.

### DISCREPANCY INDEX

In Figure 7, the discrepancy indices at stations IV, 31, 9, 10, 20, and 35 are plotted for the 1st and 2nd yr. Discrepancy values for the first 2 yr were smallest, with the least departure from the baseline, at the two most offshore stations, 20 and 35, where oil concentrations were the lowest. Moving successively shoreward, the discrepancy plots for stations 10, 9, 31, and IV slant ever more obliquely toward the vertical. Thus, these stations show progressively greater departures from the baseline demonstrating the greater faunal differences between the 2-yr period at the continuously more oiled and inshore stations. Such differences do not merely reflect the large disparities in abundance of the few most abundant species; these differences are just as applicable to the many less common species.

Enough samples were collected at stations IV, 31, and 10 in the 3rd yr so that discrepancy values could be obtained for the 1st and 3rd and 2nd and 3rd yr. Each of the three plots graphed for station 31 (Fig. 8) showed extreme departure from the baseline. Such large discrepancy indices document the considerable faunal variation at this inshore subtidal station during the 3 yr considered. A likely cause for the pronounced faunal instability was the persistently high petroleum hydrocarbon content of the sediment, which remained between 110 and 1240 mg hydrocarbon/100 g dry sediment except for the initial September 1969 sample. On the basis of these discrepancy index graphs, the fauna at station 31 achieved only the initial, incipient stages of recovery. Discrepancy indices were large for each of the three pairings for station IV, greatest for the 1st and 2nd and smallest for the 2nd and 3rd yr, indicating some recovery during the later period of study (Fig. 9).

The three discrepancy index plots at intermediately oiled station 10 (Fig. 10) remained well below the plots for the same time periods at stations IV and 31. The reduction in discrepancy values for the 2nd and 3rd yr, although modest, indicates that the fauna was in the process of stabilizing or recovering. Yet, when the discrepancy graph for the 2nd and 3rd yr at station 10 was compared with the discrepancy plots for the 1st and 2nd yr at minimally affected stations 20 and 35 (Fig. 7), it consistently yielded significantly higher discrepancy values at all densities but the smallest. We can conclude from this comparison that the fauna at station 10,

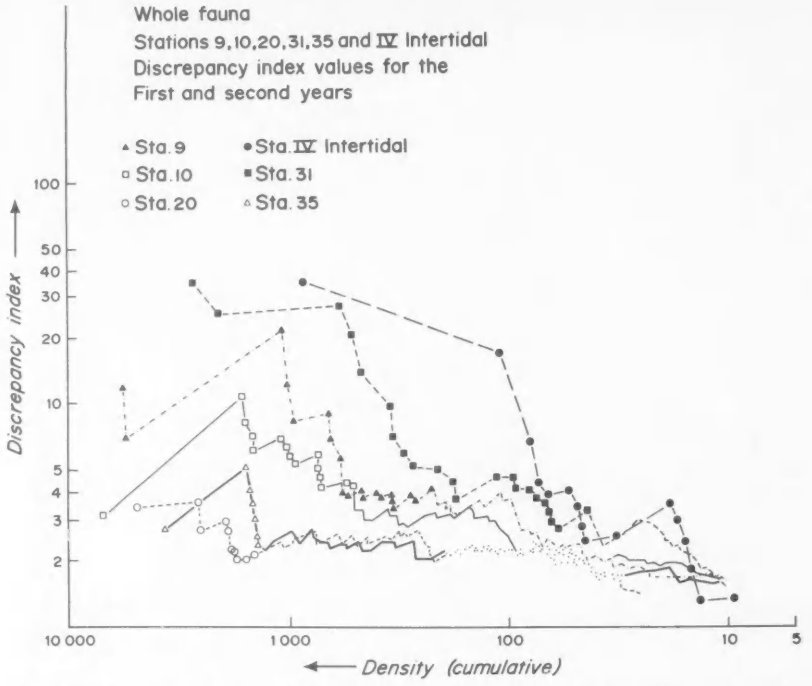


FIG. 7. Discrepancy indices obtained by comparing the composition of the fauna in the 1st with the 2nd yr of study at stations IV, 31, 9, 10, 20, and 35.

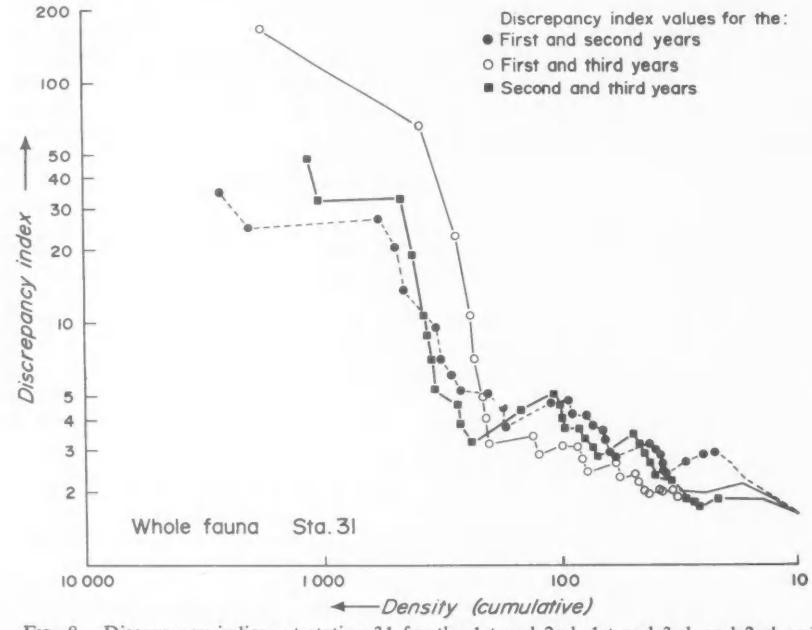


FIG. 8. Discrepancy indices at station 31 for the 1st and 2nd, 1st and 3rd, and 2nd and 3rd yr.

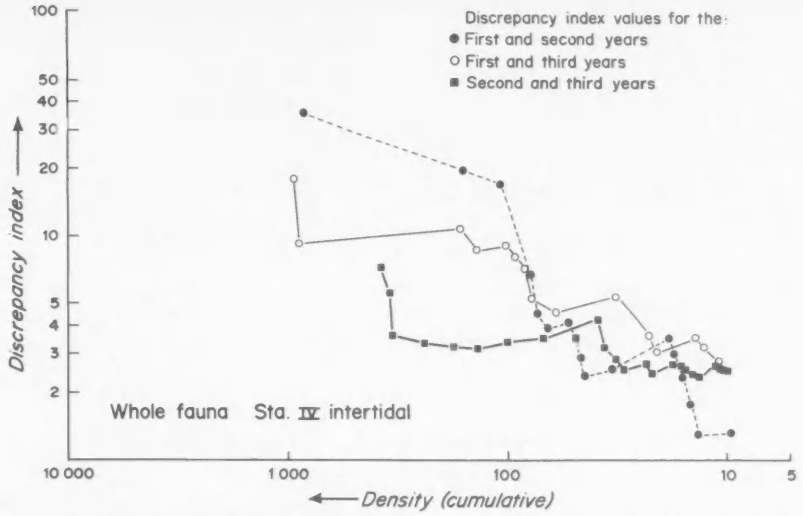


FIG. 9. Discrepancy indices at station IV for the 1st and 2nd, 1st and 3rd, and 2nd and 3rd yr.

although improving, showed only partial recovery over the 3-yr period.

## CLUSTER ANALYSIS

The bar diagram illustrated in Fig. 11 is a graphic interpretation of the stability–time hypothesis for diversity and life-history patterns among communities that evolve over a geologic time scale (Sanders 1968). It was derived from the faunal patterns that emerged from a comparative study of benthic diversity from a wide range of marine habitats. In brief, the hypothesis states that low-diversity habitats are variable and physiologically rigorous. The species found there are eurytopic, i.e. they have broad physiological tolerances. The communities present in such habitats are termed physically controlled. Alternatively, high-diversity habitats are characterized by physical conditions that have been stable and relatively uniform for long periods of time. Such minimally varying physical environments have permitted extensive biological interactions to take place with little interruption over evolutionary time. The historic results are stable and complex biologically accommodated communities composed of large numbers of stenotopic (narrow physiological tolerances) species. There is, of course, a gradual and continuous change from more biologically accommodated to more physically controlled communities along a gradient of increasing variance and severity of environmental change imposed over a geologic time scale and this is what is interpreted in Fig. 11. With the omission of the biologically accommodated and physically controlled communities from Fig. 11, it can be used to describe a profound and complementary model proposed by the late Professor R. G. Johnson to explain the short-term differences in diversity and composition within a community. The model was inspired by the findings from his detailed studies of the intertidal and subtidal benthos of Tomales Bay, Calif., during the 1960s (Johnson 1970).

Within a single biotic assemblage, there is a temporal mosaic in various stages of succession. Disturbances of the substrate by storms, freshwater runoff, radical and sudden temperature changes, etc. initiate regression trends toward lower community grades (to the right in Fig. 11). Periods of general or very local environmental stability permit succession to higher grades (to the left in Fig. 11). From the Tomales Bay research Johnson was able to show that "the order in which species entered samples of increasing diversity along stress gradients approximate the order of their appearance in succession." Lowest-grade species in a successional sequence are the most eurytopic and opportunistic. Pollution downgrades a community and severe impacts from certain pollution sources such as oil spills may completely destroy a littoral community. Recovery from such a profound perturbation will occur in a successional fashion. Employing cluster analyses, we will take a number of examples from the West Falmouth study and interpret them within the framework of the Johnson model.

However, before we undertake this exercise, some comment should be made regarding the impact of the polychaete *Mediomastus ambiseta*, the overwhelming numerical dominant at stations 9, 10, 20, 5, and 35, on the cluster analysis patterns. When the analysis is performed with and without *Mediomastus*, it becomes apparent that the presence of this species tends to blur

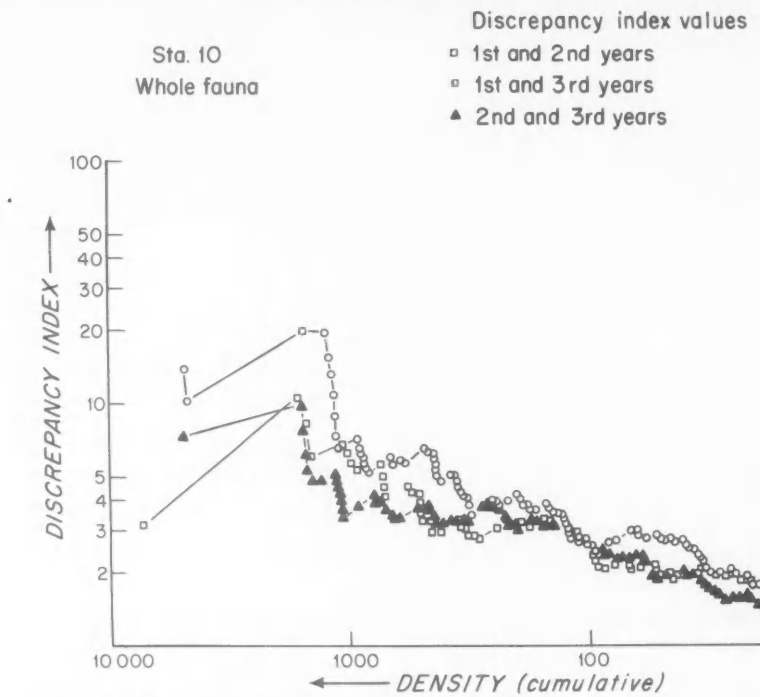


FIG. 10. Discrepancy indices at station 10 for the 1st and 2nd, 1st and 3rd, and 2nd and 3rd yr.

or obscure patterns that otherwise would be more evident (Fig. 12 and 13, Fig. 14 and 15, Fig. 16 and 17). For example, when comparing the cluster analyses for stations 10, 20, and 35 with and without *Mediomastus* (Fig. 14 and 15), the station 20 samples form a faunally more homogeneous primary cluster in the absence of *Mediomastus*. All station 35 samples are confined to a single primary cluster when *Mediomastus* is excluded. When *Mediomastus* is included, station 35 samples are present in two primary clusters. If *Mediomastus* is present in the analysis, station 10 contributes most of the samples in two faunally heterogeneous primary clusters. In the absence of this species, all station 10 samples but one are restricted to a single markedly heterogeneous primary cluster.

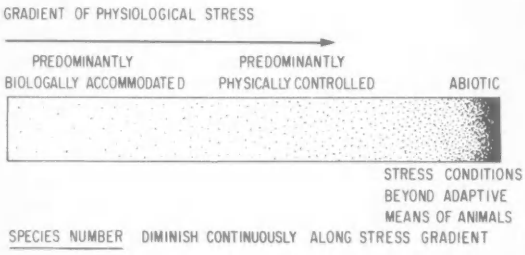
GRADIENT OF PHYSIOLOGICAL STRESS
PREDOMINANTLY BIOLOGALLY ACCOMMODATED
PREDOMINANTLY PHYSICALLY CONTROLLED
ABIOTIC
STRESS CONDITIONS BEYOND ADAPTIVE MEANS OF ANIMALS
SPECIES NUMBER DIMINISH CONTINUOUSLY ALONG STRESS GRADIENT

FIG. 11. Bar graph representation of the stability–time hypothesis.

Cluster analyses demonstrate widely differing patterns for the various stations included in this study. Certain stations, intertidal II and IV and subtidal 31, 30, 9, and 10, display profound temporal changes. Samples taken at a given station are dispersed throughout two to four primary clusters (station 10 — Fig. 12, 14, and 15; station 9 — Fig. 12, 16, and 17; stations II, IV, and 31 — Fig. 18; station 30 — Fig. 16 and 17) or if most of the samples are limited to a single primary cluster, they form a very loose, heterogeneous faunal assemblage (stations 9 and 10 — Fig. 13). Other stations, subtidal 5, 20, 35, and the intertidal Sippewissett station, are decidedly more homogeneous and are usually confined to a single primary cluster or even to a tight subgrouping within that cluster (station 20 — Fig. 14, 15, 16, and 17; station 5 — Fig. 16 and 17; station 35 — Fig. 12, 13, 14, and 15; Sippewissett — Fig. 18). The few samples from this group of stations that appear in other clusters are readily explained on the basis of highly atypical sediment composition for the August 1970 and February 1971 station 5 samples and the overwhelming numerical dominance of *Mediomastus* in the August and September 1970 station 35 samples, 93.9 and 74.1%, respectively. Faunal differences among samples from a given station are small and seasonal and

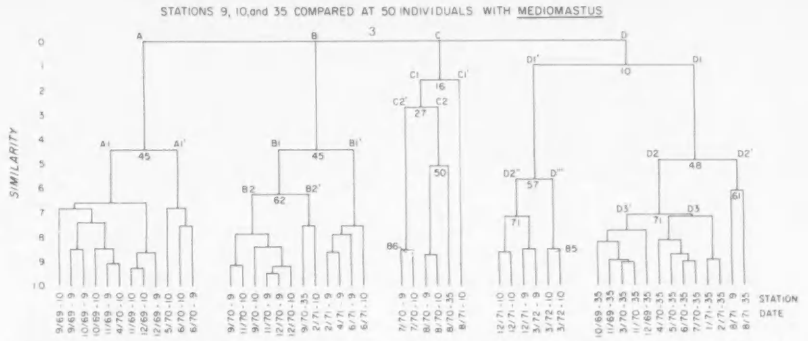


FIG. 12. Faunal similarities among samples collected at stations 9, 10, and 35 using cluster analysis.

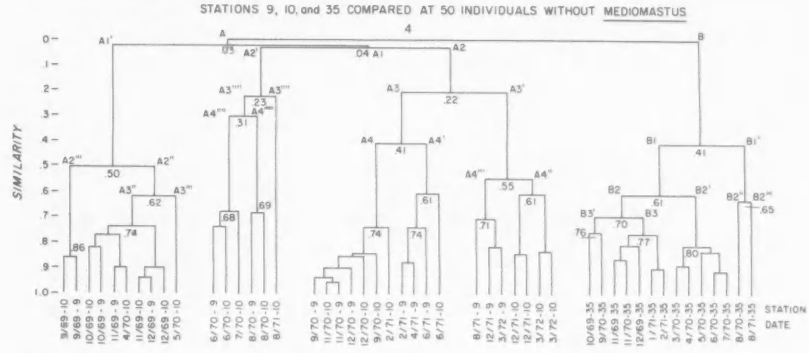


FIG. 13. Faunal similarities among samples collected at stations 9, 10, and 35 in the absence of *Mediomastus*, using cluster analysis.

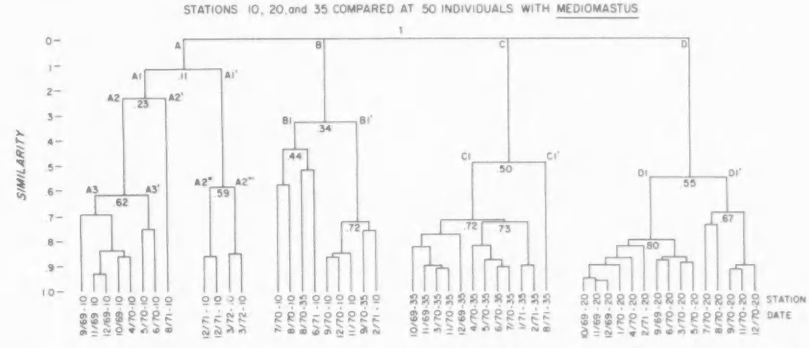


FIG. 14. Faunal similarities among samples collected at stations 10, 20, and 35 using cluster analysis.

the biota is in equilibrium rather than successional. The major seasonal differences are evident in the summer samples. The sediment composition of stations 5 and 20, with two exceptions, are essentially identical. The 23 samples of similar sediment composition are faunally interchangeable and they cluster together as a tight homogeneous subgrouping within a primary cluster.

Despite the somewhat different sediment composition, there is an almost total intermingling of the station 9 and 10 samples and the cluster patterns are temporal rather than by station. Only in the most recent samples is there a tendency for the station 9 and 10 samples to aggregate by station. Faunal changes at these two stations are rapid, large, and strongly successional rather

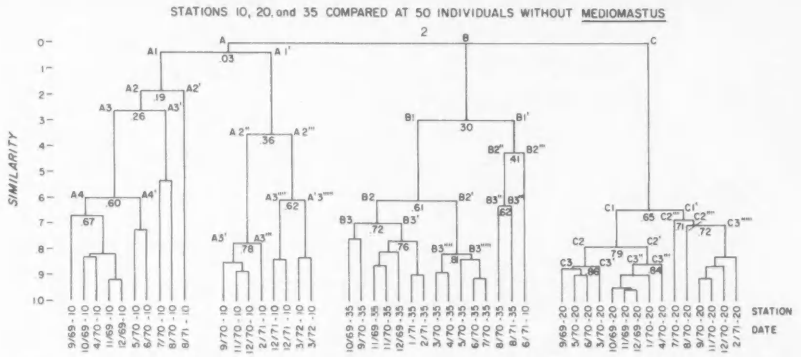


FIG. 15. Faunal similarities among samples collected at stations 10, 20, and 35 in the absence of *Mediomastus*, using cluster analysis.

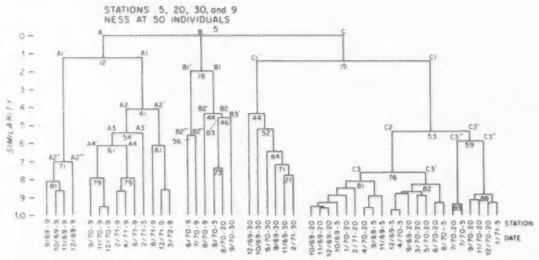


FIG. 16. Faunal similarities among samples collected at stations 5, 20, 30, and 9 using cluster analysis.

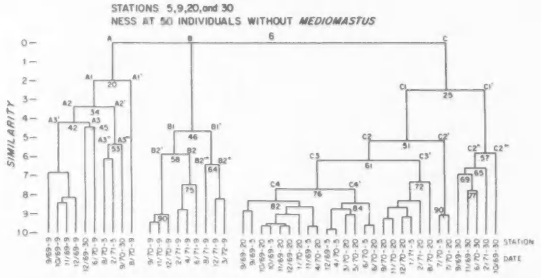


FIG. 17. Faunal similarities among samples collected at stations 5, 20, 30, and 9 in the absence of *Mediomastus*, using cluster analysis.

than seasonal, revealing the obvious nonequilibrium faunal state at stations 9 and 10 during the time period studied. Successional faunal changes move together in synchrony and cadence throughout most of the sampling interval.

Similar trends are evident for intertidal stations II and IV and subtidal station 31. Samples from stations II, IV, and 31 collected in the 11 mo following the oil spill are intimately intertwined in primary cluster A (Fig. 18), where they sort out by date rather than station. This is unexpected when one considers the profound differences in sediment composition at station 31 (mostly silt- and clay-sized particles) as compared to stations II and IV (composed of approximately 85% sand). Furthermore, stations II and IV are intertidal and station 31 is subtidal and one should expect to find significant faunal differences on this basis alone. After July 1970 there is a progressively greater tendency for samples from stations II, IV, and 31 to sort by station, although this trend never did become strong. No primary cluster in Fig. 18 is formed exclusively from either stations II, IV, or 31 samples. Instead, large successional changes that stretch through two faunally heterogeneous primary clusters at stations II and 31 and

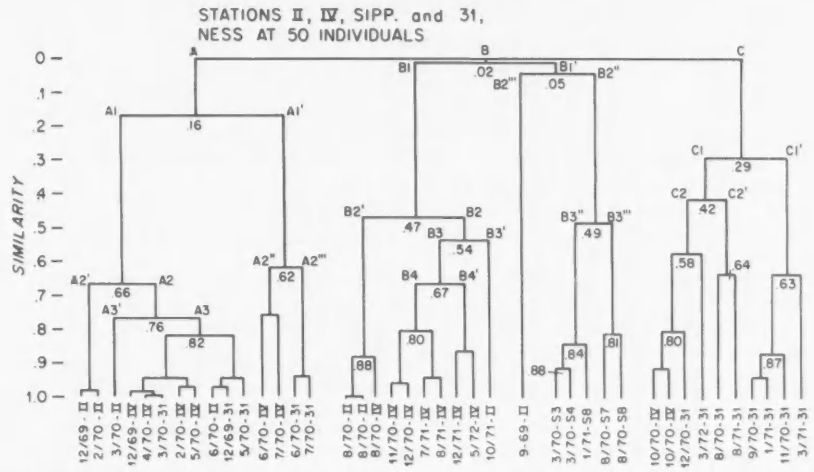


FIG. 18. Faunal similarities among samples collected at stations II, IV, Sippewissett, and 31 using cluster analysis.

three primary clusters at station IV attest to the profound faunal alterations that occurred at these stations within the time span of this study. The unoiled Sippewissett samples, on the other hand, are limited to a small subgrouping of a single primary cluster.

Each of the stations that was markedly affected by the oil spill, intertidal stations II and IV and subtidal stations 9, 10, 30, and 31, displayed a continuous and rapid faunal change in the absence of recurring seasonal patterns. Each of them documents a classic example of succession in response to a severe stress, and their faunal patterns support the thesis that the more opportunistic species forming the earlier successional stages are less discriminating regarding environmental features such as sediment composition than are species present during equilibrium conditions. Stations that were either marginally affected or unaffected by the 1969 *Florida* oil spill, 5, 20, and 35 and the intertidal Sippewissett station, maintained a stable faunal equilibrium throughout the sampling period. These results are exactly what would have been predicted from the Johnson model.

## Summary

The September 1969 *Florida* oil spill in Buzzards Bay resulted in a massive and immediate kill of intertidal and subtidal fauna in the Wild Harbor area of North Falmouth, Mass. Our long-term research objective was to occupy certain stations along a gradient of diminishing stress from very heavy to very lightly or even unoiled sites. By reoccupying each station at monthly or bimonthly intervals, changes in oil concentration and composition were carefully monitored and related to the detailed analyses of the concomitant biological samples.

Shortly after the nearly total eradication of the benthic fauna at the severely oiled intertidal and subtidal stations, these bottoms were occupied by the opportunistic polychaete *Capitella capitata*, which explosively increased in numbers to form essentially unispecies cultures at densities from tens of thousands to more than 200 000 individuals/m$^2$. After about 7 mo at these highly elevated densities, *Capitella* populations crashed and were replaced by a few less opportunistic species. Throughout the subsequent 30-mo sampling interval there were abrupt and marked changes in faunal composition, and densities fluctuated widely from one sampling date to the next. The Sippewissett Marsh control samples displayed considerably more stability regarding densities, species numbers, and faunal composition. These findings strongly support the interpretations that the differing temporal patterns at the Wild Harbor River and Sippewissett stations were due to the oil-induced stress in the Wild Harbor estuary.

For nearly a year after the oil spill, densities and species numbers at the intermediately oiled subtidal stations in the outer reaches of Wild Harbor were markedly depressed. In August 1970 there was a huge settlement of very young individuals from the overlying water onto these "biologically undersaturated" bottoms that resulted in 20–30-fold density increases and 2.4–3.4 enrichment of species. This event, in turn, was followed by a continuous and precipitous reduction in densities and species throughout the 2nd yr of study. Higher species numbers and less density and species variation during the 3rd yr were indicative of some recovery.

Unlike either the severely or intermediately oiled sites, the minimally oiled stations farther offshore showed little variation in species composition and much smaller density variations throughout the sampling period.

Few species were present at the severely oiled stations in half or more of the samples. A greater number ap-

peared in samples collected at the intermediately oiled localities, although very few of them were found in 90% or more of the samples. The still more numerous high-fidelity species at the minimally oiled stations and the significantly greater number of species occurring in every sample are indicative of the faunal stability that persisted at these sites throughout the sampling period.

Coefficient of variation measurements for the total sampling interval demonstrate much lower faunal variabilities for the stations at the minimally affected end of the oil-induced stress gradient than at the intermediately oiled localities and decisively lower variabilities than those observed for the highly unstable faunal regimes found at those stations that were severely oiled.

The discrepancy index, which was developed to measure average yearly differences in the benthic fauna at a given station, reveals that only small faunal differences were evident between the 1st and 2nd yr of study at the minimally oiled stations, while the faunal differences were very great at the intermediately and severely oiled stations. The severely oiled stations, with persistently high petroleum hydrocarbon concentrations, continued to show profound yearly differences between the 3rd yr and either of the previous 2 yr. Faunal differences between the 2nd and 3rd yr of study were smaller at intermediately oiled station 10 than the differences found for the 1st and 2nd and 1st and 3rd yr, indicating that the fauna at this station was in the process of stabilizing. Yet, faunal differences between the 2nd and 3rd yr were significantly greater than any of the values measured at the minimally oiled localities demonstrating only partial recovery at station 10 over the 3-yr period.

Cluster analysis techniques that documented unidirectional, continuing, and rapid faunal changes at both the initially severely and intermediately oiled stations in the absence of recurring seasonal patterns reveal a classic example of succession in response to a severe stress. The more opportunistic species present in the earlier successional stages were less discriminating in regard to environmental parameters such as sediment composition than were species that were found during the later successional stages. The minimally oiled and unoiled stations, in contrast, maintained an equilibrium, stable faunal pattern throughout the sampling period.

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# No. 2 Fuel Oil Spill in Bourne, Massachusetts: Immediate Assessment of the Effects on Marine Invertebrates and a 3-Year Study of Growth and Recovery of a Salt Marsh[1,2]

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HAMPSON, G. R., AND E. T. MOUL. 1978. No. 2 fuel oil spill in Bourne, Massachusetts: immediate assessment of the effects on marine invertebrates and a 3-year study of growth and recovery of a salt marsh. J. Fish. Res. Board Can. 35: 731–744.

On October 9, 1974 the oil barge *Bouchard 65* loaded with 73 000 barrels of oil spilled what was initially thought by the Coast Guard to be a few barrels and later raised to an undetermined amount of No. 2 fuel oil off the west entrance of the Cape Cod Canal in Buzzards Bay, Massachusetts (anchor site C, Fig. 1). Within the following 2-wk period, oil from the barge was found contained along the west side of Bassett's Island and inner Red Brook Harbor, a distance of 5.0 km from the site of the spillage. Qualitative samples of dead and moribund marine invertebrates were collected in tide pools and slight depressions along the beaches. A collection consisting of 4360 invertebrates comprising 105 species, plus 2 species of fish were found in 8 samples. Noticeable effects of the oil on the salt-marsh plant community were also observed. A detailed quantitative examination was begun to determine the effects of the oil on various components of the affected salt-marsh community in Winsor Cove compared to a selected control site. From data collected in September 1977, the marsh grass in the lower intertidal zone in Winsor Cove has shown an inability to reestablish itself by either reseeding or rhizome growth. The associated sediments show a correspondingly high concentration of petroleum hydrocarbons impregnated in the peat substrate. Erosion rates measured in the affected area, as a result of the 3-yr period of marsh degeneration, were 24 times greater than the control site. Microscopic algae were collected during the sampling period and those present were considered least sensitive to environmental changes. Examination of the interstitial fauna found in the study area in the summer of 1977 showed an extremely reduced number of individuals and species.

*Key words:* salt marsh, No. 2 fuel oil, petroleum, erosion, pollution, algae, marsh grass, interstitial fauna, marine invertebrates

HAMPSON, G. R., AND E. T. MOUL. 1978. No. 2 fuel oil spill in Bourne, Massachusetts: immediate assessment of the effects on marine invertebrates and a 3-year study of growth and recovery of a salt marsh. J. Fish. Res. Board Can. 35: 731–744.

Le 9 octobre 1974, le chaland-citerne *Bouchard 65* portant une charge de 73 000 barils de pétrole déversa ce que la Garde côtière crut d'abord être seulement quelques barils mais qui s'éleva par la suite à une quantité indéterminée de fuel-oil nº 2 au large de l'entrée occidentale du canal de Cape Cod dans la baie Buzzards, Massachussets (poste d'ancrage C, fig. 1). Durant les 2 sem qui suivirent, le pétrole s'était répandu le long de la côte occidentale de l'île Bassett's et le port intérieur de Red Brook, une distance de 5.0 km du site du déversement. Nous avons recueilli des échantillons qualitatifs d'invertébrés marins morts et mourants dans des étangs à marées et de petites dépressions le long des plages. Dans 8 échantillons, nous avons trouvé une collection de 4360 invertébrés comprenant 105 espèces, plus 2 espèces de poissons. Nous avons de plus observé des effets notables du pétrole sur la communauté végétale du marais salé. Nous avons entrepris un examen quantitatif détaillé dans le but de déterminer les effets du pétrole sur les divers composants de la communauté du marais salé affecté de Winsor Cove comparative-

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[2]Woods Hole Oceanographic Institution Contribution No. 4088.

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ment à un site témoin. Les données recueillies en septembre 1977 indiquent que l'herbe du marais dans la zone intertidale inférieure de Winsor Cove a été incapable de se rétablir soit par réensemencement, soit par croissance des rhizomes. Les sédiments qui lui sont associés montrent une teneur élevée correspondante en hydrocarbures de pétrole dont le substrat de tourbe est imprégné. Comme résultat de 3 a de dégénération du marais, les taux d'érosion mesurés dans la région affectée sont 24 fois supérieurs à ceux du site témoin.

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THE oil barge *Bouchard 65* was damaged by an unknown submerged object at the west entrance of Buzzards Bay on October 9, 1974. In an attempt to off-load the remaining cargo, the barge was towed to the west of the canal to anchorage site C at the entrance to Hog Island Channel (Fig. 1). Rough seas prevented boom containment of the oil and on October 10 the resultant oil slick headed in a northeasterly direction where some of the oil came ashore on Bassett's Island. Strong southeast winds then drove most of the oil into the confines of Bassett's Island and Red Brook Harbor (Fig. 1) where the oil remained on the surface for approximately 1 wk–10 d. As is often the case, oil containment both at sea and on land was ineffective due to excessive wind conditions and lack of proper organization of the clean up operation. Consequently, in Winsor Cove (Fig. 1) oil and oil-soaked seaweed churned in the swash zone within 2–3 d.

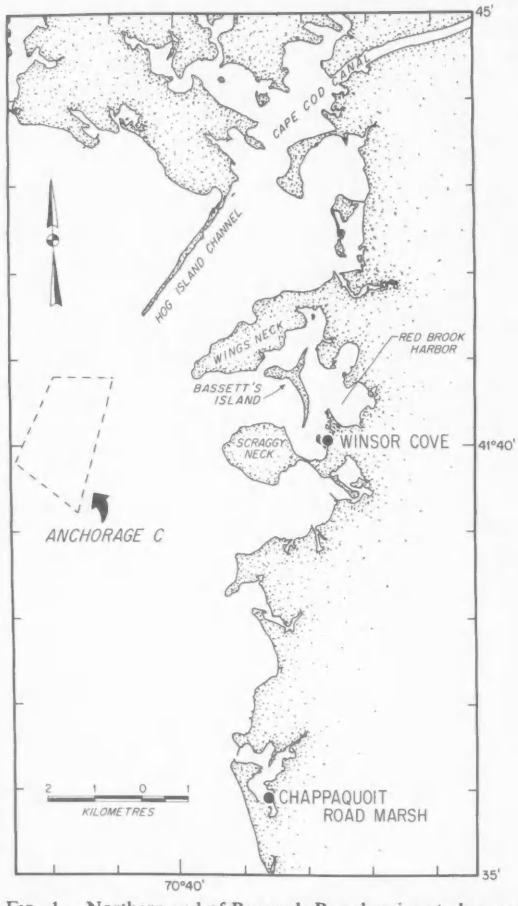


FIG. 1. Northern end of Buzzards Bay showing study area.

October 12 witnessed the first signs of massive mortalities in several locations around Bassett's Island and Winsor Cove. Sea worms, *Nereis virens*, were observed on the bottom in a moribund state, and small gastropods were lying on the bottom with apertures facing upward. Occasional decapods including fiddler crabs, *Uca pugnax*, were interspersed with the oiled-covered seaweed.

The physical configuration of the west side of Bassett's Island formed a natural containment for the incoming oil which coated the entire intertidal zone of the west side of the island substantially. Consequently, the mortalities witnessed along the westerly side of the island were generally more severe than those found on the easterly side. Along the southwest channel between Scraggy Neck and Bassett's Island noticeable quantities of the surf clam, *Spisula solidissima*, approximately 3–4/m$^2$, were on the sediment surface with their valves gaping. Also, a number of razor clams, *Ensis directus*, protruded from their burrows while other individuals were lying laterally on the bottom. Normal escape responses of most of the moribund invertebrates were lacking. Large numbers of dead polychaetes, molluscs, and crustaceans were found accumulated in slight depressions in the sand and along the strand line. A survey conducted at Bassett's Island on October 13, 1974 indicated that the mortality was probably at a maximum and that the whole spectrum of the invertebrate fauna was affected. On the same day, the first signs of extensive mortalities of crustaceans, molluscs, and polychates were noted in Winsor Cove where strong northeasterly winds formed an obvious emulsion of oil and seawater which aided the transport of oil into the subtidal sediments.

By October 17, 1974 most of the noticeable damage to the macrobenthos at Bassett's Island was no longer discernible due to decomposition and current transport of the soft-bodied animals. The heavier shells from freshly decomposed bivalves and carapaces of decapods and other crustaceans remained in the sand depressions as evidence of the damage.

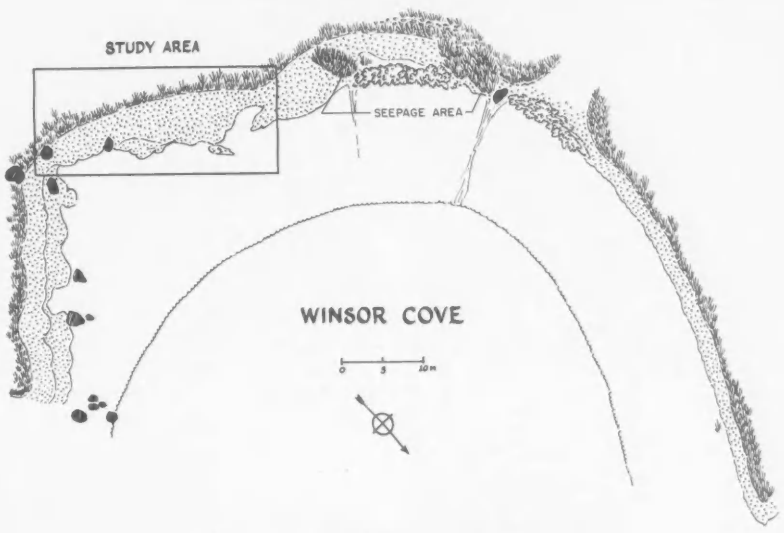


FIG. 2. Winsor Cove showing study area.

## Methods

To determine subtidal effects of the spill, a series of scuba dives was made on October 17, 1974 along a transect starting at a point approximately 900 m off Wings Neck Point and running towards the intertidal zone off Bassett's Island.

Eight qualitative benthic samples were collected from Bassett's Island and Winsor Cove between October 12 and 14, 1974. These collections were washed through a screen (aperture = 0.297 mm) and preserved for future identification, from which a species list was eventually produced (Appendix 1). No attempt was made to obtain quantitative samples because of the nature of the coarse beach sediments, the artificial aggregations of animals found in the sediment depressions, and the obvious disruption of the animals from their natural habitats. A total of 4360 invertebrates comprising 105 species and 2 species of fish were collected.

In Winsor Cove and off Chappaquoit Road a series of quadrats for plant counts were established running perpendicular from low water to the high tide level (Fig. 2, 3). The boundary of each quadrat was delineated by the perimeter of an aluminum frame 41 × 35 cm (0.14 m²). Successive quadrats were located by a series of wooden stakes driven into the marsh with approximately 10 cm exposed. Therefore, exact replication of each plot was possible for future plant counts.

Unfortunately, quantitative comparative species data from both marsh stations were not obtained until July 1975. However, precise photographic stations were established with permanent markers in Winsor Cove on October 23, 1974 which documented the extensive coverage of *Spartina alterniflora* and *Salicornia* before decomposition. Within each spot, the species and individual stem counts of the marine grasses were recorded each year, once in the late spring and again in early fall for the period July 15, 1976 to September 7, 1977. When individual branchings of *Salicornia* and *Fucus* became too numerous to count, our findings were quantified in terms of number of clumps or percent cover of available space within the quadrat. Although not as precise, this method provided a general comparative measure of density of *Salicornia* and *Fucus* which would have otherwise been impossible to obtain by stem counts.

The Chappaquoit Road station (Fig. 1, 3) was carefully selected to serve as a valid control to the affected area regarding floral similarity, location of marsh to prevailing wind direction, and maximum protection from wave erosion. The control transect was arranged differently in that all the quadrats were not adjacent, primarily due to the existence of a more gradual marsh slope. In some cases quadrats were spaced apart within a floral community to study a wider range of vegetation.

In addition, on each sampling date at Winsor Cove and Chappaquoit qualitative surface scrapings of the peat surface within the transect area were taken for microscopic algae which were later identified under the microscope.

On July 27, 1977 replicate shallow cores (2 × 2 × 4 cm depth) were taken in Winsor Cove and the control marsh to quantify the relative abundance of interstitial fauna found living in one zone of the marsh. A site adjacent to quadrat no. 7 was chosen in Winsor Cove in which a total of four cores were collected. The samples were washed through a 0.1-mm screen, and stained and sorted under the microscope. The control was treated similarly; however, only two cores were sorted.

In October 1974, at both Winsor Cove and Chappaquoit marshes, the boundary between the marsh and intertidal zone was profiled with a series of stakes placed approximately 1 m apart to delineate the original marsh boundary. Our intent was to measure the erosion of the peat along the edge of the marsh. The total length of marsh marked for erosion studies in Winsor Cove was 17.4 m and for Chappaquoit, 13.0 m.

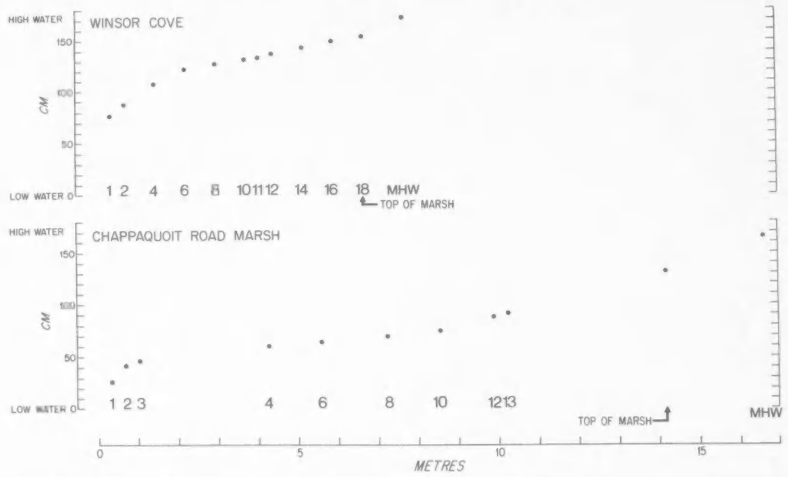


FIG. 3. Comparative marsh profiles showing positions of the quadrats in the intertidal regions of Winsor Cove and Chappaquoit Marshes.

## Results

From the scuba dives conducted on October 17, 1974 no apparent damage to the macrobenthos was seen in the deeper subtidal zone (5–8 m depth). Visible mortalities of larger subtidal animals appeared to be restricted to the area off the west shore of Bassett's Island from the intertidal zone out to a depth of 2.0–2.5 m. Within this depth interval, occasional fragments of dead invertebrates and freshly gaping bivalves could be seen at the bottom.

On a visit to Winsor Cove on October 18, 1974 (8 d after the spill), we found an occasional dead or moribund *Uca pugnax* in or around the opening to its burrow in the peat. Also seen were the mud snails *Nassarius obsoletus* and *Littorina littorea* lying aperture side up. The soft-shell clam, *Mya arenaria*, and the quahog, *Venus mercenaria*, and other species of bivalves were lying on the sediment surface with shells gaping and siphons extended. When probed, these bivalves exhibited little or no retraction response. The remains of other soft-bodied carcasses were no longer evident due to decomposition which was observed in the field.

By October 23, 1974 we observed that the more noticeable marsh plants (*Spartina*, *Salicornia*, *Limnonium*) in Winsor Cove exhibited the same brown discoloration associated with the No. 2 oil as witnessed in the West Falmouth spill of 1969 (Sanders et al. 1977). In contrast, a visit to a nearby marsh at Chappaquoit in West Falmouth showed no evidence of dying shoots and discoloration. At this point we decided to undertake an intensive study of the long-term effect of No. 2 fuel oil on the marsh in Winsor Cove. Several components of the salt marsh were taken into consideration: vegetation, from algae to higher plants, erosion and hydrocarbon content of the peat, and interstitial fauna were surveyed.

Photographs taken October 1974 (Fig. 4) show an overview of the transect area in Winsor Cove. These photographs clearly illustrate the density of marsh grasses growing close to the water's edge on the Winsor Cove transect before the marsh degenerated. Photographs of the same sites taken 3 yr later in September 1977 (Fig. 5) show the total absence of the *Spartina* stand in the lower marsh zone. Also, a quadrat established in the field that incorporated a stand of *Salicornia virginica* was photographed on October 23, 1974 (Fig. 6a) and again on September 9, 1977 (Fig. 6b), which clearly shows little evidence of regeneration of high plants. An artist's rendition of before and after was drawn from composite photographs taken in October 1974 and September 1977 which illustrate the extent of the recession of the marsh over the 3-yr period (Fig. 7).

During the same period, two photoquadrats were also established in the Chappaquoit control marsh. Although not shown in this manuscript, these sites exhibited a healthy stand of *Spartina* and *Salicornia* during the entire period observed. Figure 8 compares the species composition of each quadrat within the transects over the entire sampling period for Winsor Cove and Chappaquoit (control). These data show little reestablishment of the marsh in the lower or first 11 quadrats either by reseeding or rhizome growth. In the spring of each year a few seedlings or small stems of *Salicornia*, *Spartina*, *Limonium*, and *Fucus* had appeared, but by late summer, the majority of the new recruits was either absent or remained as seedlings or dwarfed specimens. *Salicornia virginica*, however, exhibited a certain amount of recovery in the higher marsh quadrats after September 1976 (see quadrats 12–14, Fig. 8a). Generally the lower quadrats appeared to be subjected to a continual

FIG. 4. Winsor Cove. a, Overview of upper and mid-marsh, October 1974; b, view of transect site, October 1974; c, quadrat no. 1, October 1974.

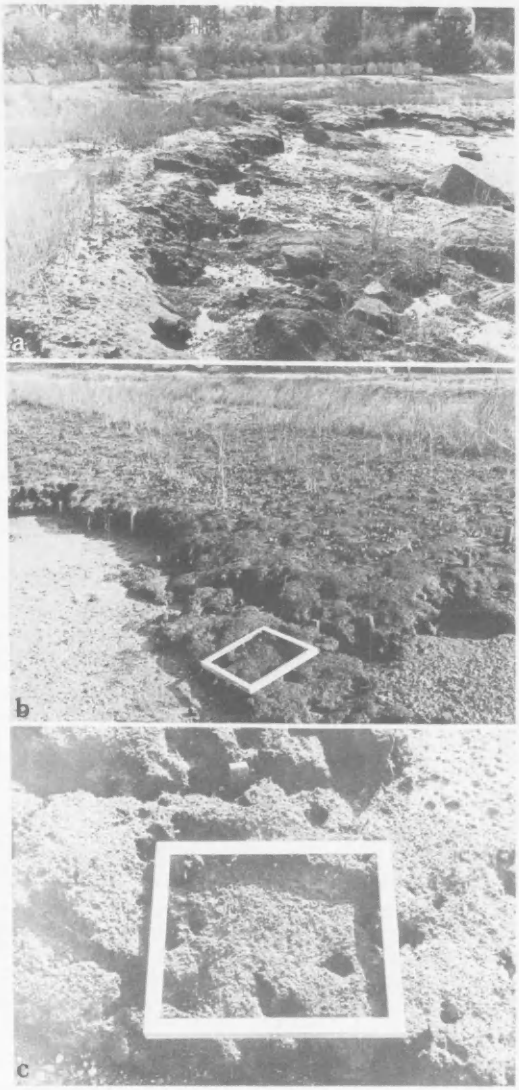


FIG. 5. Winsor Cove. a, Overview of upper and mid-marsh, September 1977; b, view of transect, September 1977; c, quadrat no. 1, September 1977.

stress that retarded or restricted plant growth and regeneration.

The common ribbed mussel, *Modiolus demissus*, appeared to be one of the few organisms that successfully recovered from the spill. Observations made of *Modiolus* in Winsor Cove within a few months after the spill showed most of the valves to be gaping and empty. However, counts obtained from the quadrats in the spring and fall of 1976 and 1977 (2 and 3-yr after the spill) showed that Winsor Cove had 3.4 times the density of *Modiolus* found in the control site. The distribution pattern was similar in both areas in that the majority of individuals was concentrated in the mid- and upper marsh. However, in Winsor Cove the ribbed mussel was absent in the first two quadrats throughout the study period, and in Chappaquoit, a total of 14 individuals was counted in the first two quadrats during 1976 and 1977. These data suggest that *Modiolus*, acting

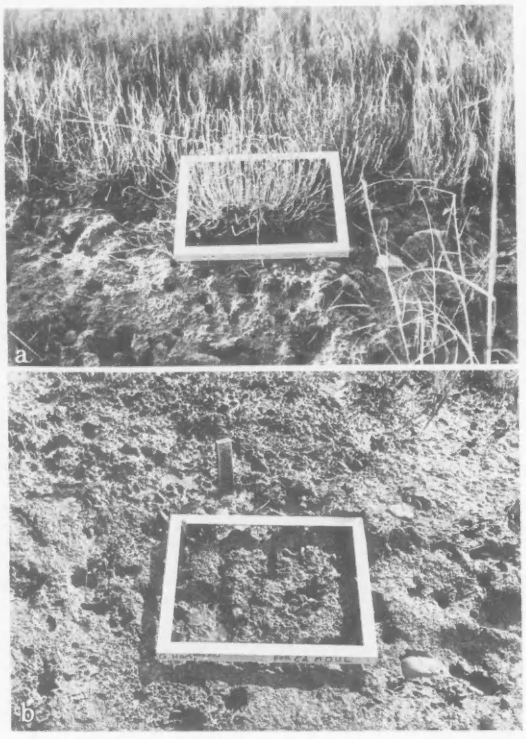


FIG. 6. Winsor Cove. a, *Salicornia virginica* stand, October 1974; b, *Salicornia virginica* stand, September 1977.

as an opportunistic species, effectively repopulated a biologically unsaturated area as existed in Winsor Cove from 1975 to 1977. In the absence of an extensive *Spartina* root system, *Modiolus* was unrestricted in space and habitat.

## SEEPAGE AREA

At Winsor Cove in a few isolated areas at the edge of the marsh, water seepage was observed to pass continually over the sediments during low tide (Fig. 2, 9). The roots of these plants were continually flushed with surface water that prevented the plants and sediments from becoming markedly impregnated with oil. It is noteworthy that the surface sediment in the seepage area was sandy mud and coarse gravel at the surface. Also, the strong odor of oil found in the other sediments and peat at Winsor Cove was lacking in this seepage area. In this zone, *Spartina alterniflora* growth was only marginally affected by the spill and growth exceeded that found elsewhere in the Winsor Cove area (Table 1).

## *Spartina alterniflora* GROWTH MEASUREMENTS

To observe the effect of oil on growth, a series of comparative random height measurements was made

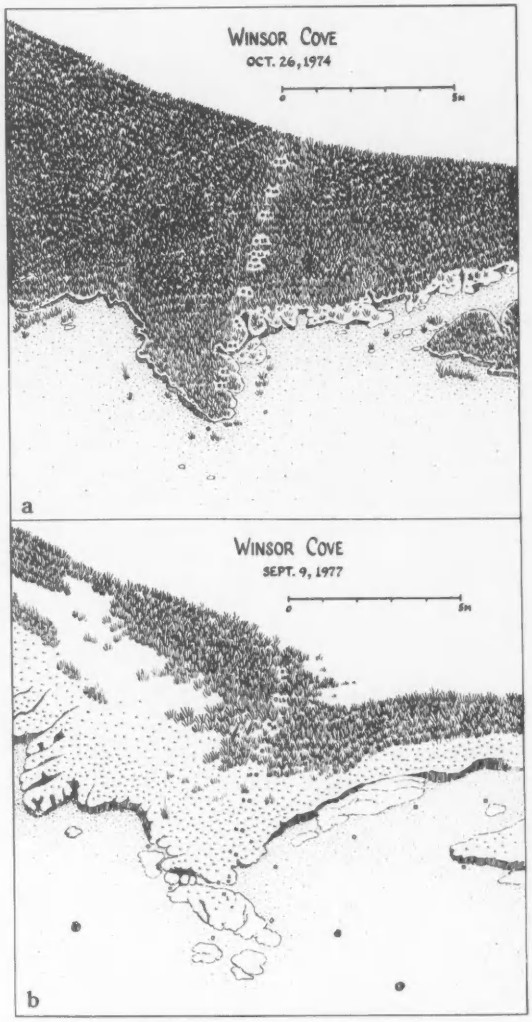


FIG. 7. Artist's rendition of quantity of marsh present at Winsor Cove site (a) October 23, 1974 and (b) September 9, 1977.

between September 1976 and 1977 of *Spartina alterniflora* stems growing in Winsor Cove (including the seepage area) and in the control in West Falmouth (Table 1). The Winsor Cove Marsh was roughly divided into an upper and lower marsh as determined by the noticeable boundary between highly stressed plant growth in the lower zone found within quadrats 1–11 (Fig. 8a) and the somewhat more productive higher zone between quadrats 12–18. The control station was also divided into a comparative upper and lower marsh zone and measurements were collected on similar dates.

Results from the 3-yr study in Winsor Cove generally

TABLE 1. Comparative stem measurements of *Spartina alterniflora* from an oil-affected area (Winsor Cove) and a control area (Chappaquoit Marsh).

| | Total stems in quadrats | Mean height (cm) | Total stems measured | Mean stem no./quadrat |
|---|---|---|---|---|
| Chappaquoit Marsh | | | | |
| lower marsh (quadrats 1–4) | | | | |
| Sept. 16, 1976 | 109 | 80 | 9 | 27.3 |
| Sept. 7, 1977 | 133 | 84 | 14 | 33.3 |
| upper marsh (quadrats 6, 8, 10, 12) | | | | |
| Sept. 16, 1976 | 72 | 59 | 26 | 18.0 |
| Sept. 7, 1977 | 110 | 60 | 9 | 27.5 |
| Winsor Cove | | | | |
| lower marsh (quadrats 1–11) | | | | |
| Sept. 7, 1976 | 7 | 8.3 | 7 | .64 |
| Sept. 7, 1977 | 5 | 30.2 | 15 | .45 |
| upper marsh (quadrats 12–18) | | | | |
| Sept. 7, 1976 | 6 | 33 | 43 | .85 |
| Sept. 7, 1977 | 7 | 70 | 19 | 1.0 |
| seepage area | | | | |
| Sept. 7, 1976 | — | 126 | 20 | — |
| Sept. 7, 1977 | — | 147 | 19 | — |

showed that plants found growing on the upper slope were larger and slightly more abundant than the same species in the lower zone. The greatest difference between these two zones was found in mean stem height, where a 2–4 fold increase was measured in the high marsh over the low marsh in Winsor Cove (Table 1).

In 1976 with the exception of the seepage area, data from the control site consistently showed a greater *Spartina* stem height and count in both marsh zones compared to the affected area. However, by 1977, the heights of the upper marsh in both locations were similar.

### MICROSCOPIC ALGAE

At Winsor Cove a blue-green algal mat was present and extended from quadrats 1 to 10 (a distance of 3.5 m). Nine species of blue-green algae were identified over the period July 1975 to September 1977. Only two species were present in the first collection and over the collecting period never more than five species were observed at any one time. Algae present were a species of *Vaucheria* and several diatoms, *Melosira sulcata* the most common species. Only one species of blue-green algae, *Microcoleus chthonoplastes*, was found in all collections and at all levels of the marsh where the algal mat occurred. *Vaucheria* was present at the seaward edge of the marsh in quadrat no. 1 in late summer. *Rivularia atra* appeared at the lower edge of the marsh in September 1976 and by September 1977 it was abundant from quadrat no. 1 to no. 6. The blue-greens that occurred in most collections are the species considered robust and least sensitive to environmental changes (Stewart and Pugh 1963). These included *M. chthonoplastes*, *Lyngbya aestuarii*, and *L. confervoides*. All other blue-greens recorded for the marsh at Winsor Cove were ephemeral.

### MACROSCOPIC ALGAE

In the shallow water's edge of Chappaquoit Marsh, macroscopic algae were always present in great abundance. *Ascophyllum nodosum*, *Fucus vesiculosus*, *Vaucheria spiralis*, *Enteromorpha* spp., and *L. majuscula* were the common species. *Enteromorpha plumosa* was also found growing in July 1975 and September 1977; *E. glaucensens* was recorded in the July 1977 sampling period (Fig. 8b).

At Winsor Cove these algal species were rarely found at the water's edge or in the quadrats. One unidentified species, *Enteromorpha* sp., was found sparingly in September 1976 and 1977, and *E. linza* appeared in the eighth quadrat in the September 1977 sampling period (Fig. 8a).

### EROSION STUDIES

Profile comparisons obtained between October 1974 and August 1977 demonstrated an average erosion of 0.64 cm for Chappaquoit control and 15.6 cm for Winsor Cove (Fig. 10). In other words, the rate of erosion at Winsor Cove was 24 times that found at Chappaquoit.

### INTERSTITIAL FAUNA

The four Winsor Cove cores collected for interstitial analysis had a total of 21 specimens that included nematodes, harpacticoid copepods, oligochaetes, and Diptera larvae. In contrast, a total of 261 individuals from two

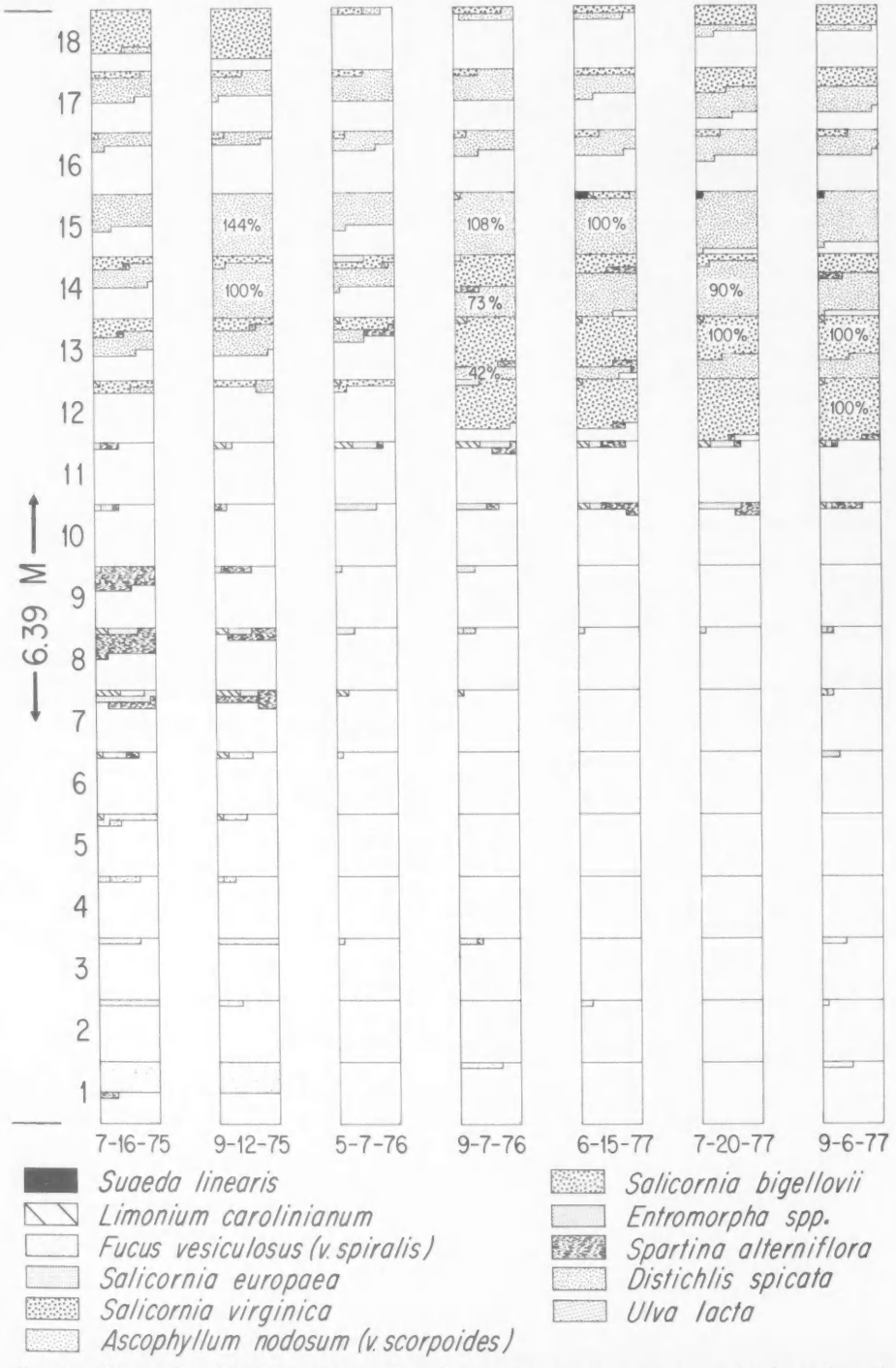


FIG. 8a. Winsor Cove Marsh sampling quadrats showing percent coverage of vegetation for each quadrat over time.

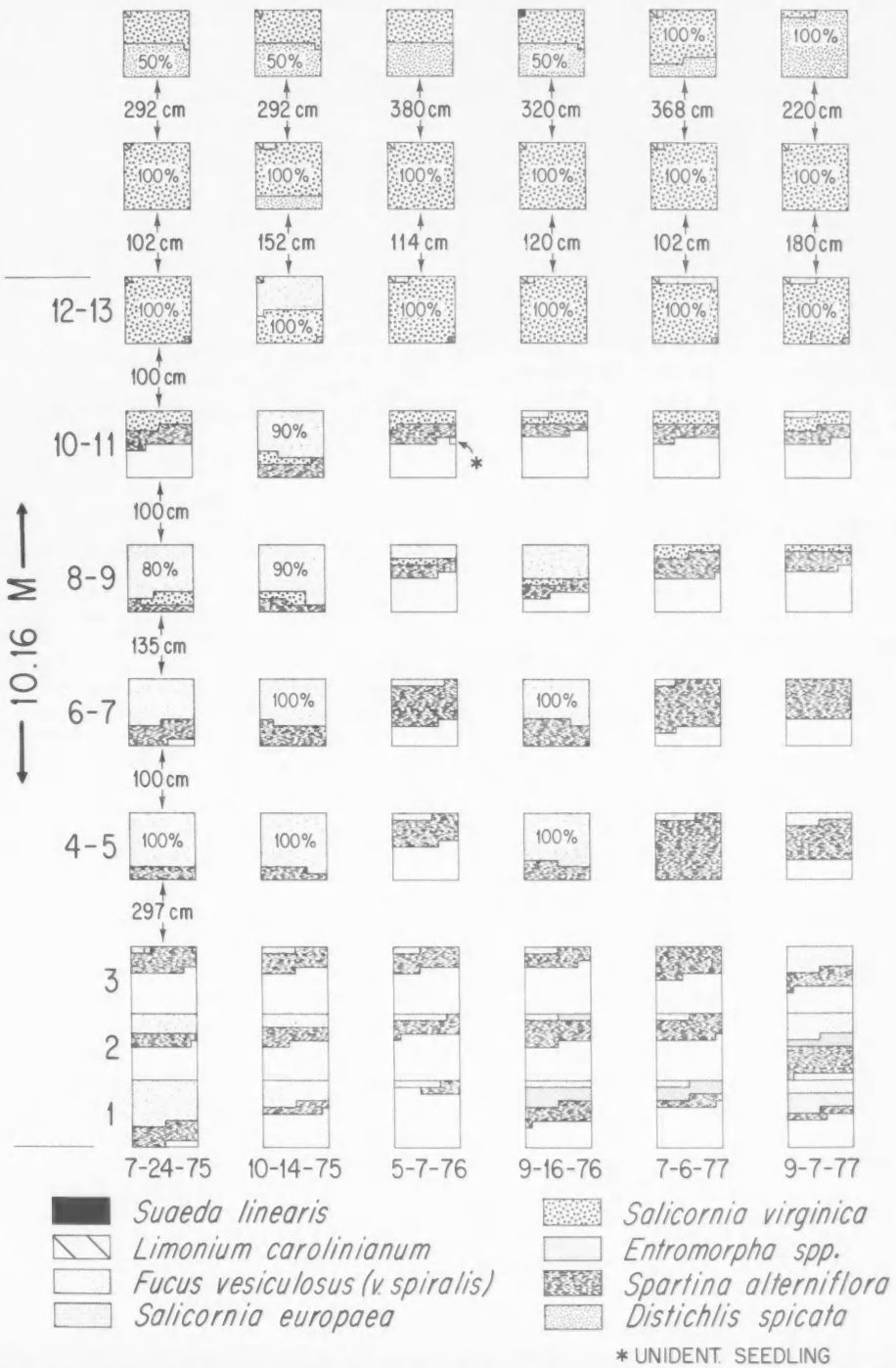


FIG. 8b. Chappaquoit Marsh sampling quadrats showing percent coverage of vegetation for each quadrat over time.

FIG. 9. Seepage area, Winsor Cove, September 1977.

cores was found in the Chappaquoit Marsh samples which included the previously mentioned groups plus a species of tanaid and two species of polychaetes. The interstitial fauna found in Winsor Cove Marsh was extremely reduced and correlated with the high concentration of oil existing in the peat sediments. These conclusions were sustantiated by Dr J. J. Lee (personal communication 1977) of City College of New York, after a site visit to the area in August 1977. Chappaquoit control was also sampled for petroleum hydrocarbons in May 1975 and no significant levels of petroleum hydrocarbons were detected; however, samples obtained in July 1977 in the control area revealed a noticeable envelope of "weathered" petroleum in the sediments. The source of this influx into the area was unclear. Although the total weight of alkanes and cycloalkanes fraction was relatively high, it was discovered from gas-chromatographic analysis that the value for the light aromatic fraction was unchanged compared to the May 1975 value.

### HYDROCARBON ANALYSIS

Analysis of petroleum hydrocarbon concentrations found in the sediments adjacent to the transect in Winsor Cove revealed a high oil concentration in the salt-marsh peat in May 1975 and July 1977 (Teal et al. 1978).

Complete hydrocarbon analysis of sediments taken from Winsor Cove during the same period of this study has been performed by Dr J. Farrington and Mr B. Tripp of the Woods Hole Oceanographic Institution. Their results are presented elsewhere in these proceedings (Teal et al. 1978).

## Discussion

### PETROLEUM HYDROCARBONS VS. PLANT MORTALITY

Analysis for petroleum hydrocarbon concentrations found in sediments in the study area of Winsor Cove revealed a high oil concentration in the salt-marsh peat (Teal et al. 1978). Wherever the marsh revealed signs of acute mortality, a strong odor of oil was detected in the sediments.

Baker (1970) states that "oils vary in toxicity according to content of low boiling compounds, unsaturated

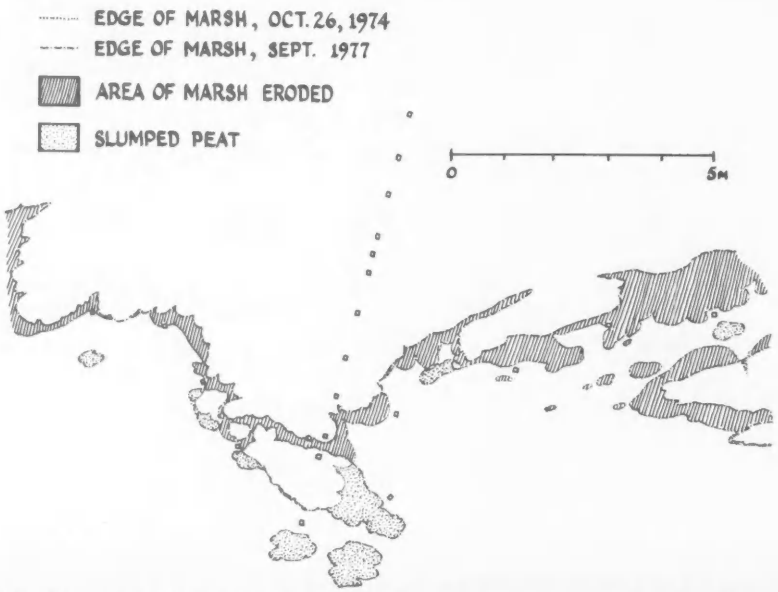


FIG. 10. Winsor Cove showing amount of erosion and slumping over a 3-yr period.

compounds, aromatics and acids." Greatest acute damage is generally caused by the "very toxic oxidized oils and aromatics which stop respiration and cause widespread injury and death"; the total severity being dependent on the constituents and amount of the oil, the environmental conditions, and the species of plant involved.

A study by Burk (1977) discusses a 4-yr analysis of vegetation following a fuel oil spill in a freshwater marsh off Mill River in Northamton, Massachusetts. The study shows analogous similarities to Winsor Cove in long-term marsh destruction. Eighteen of the 45 plant species found before the spill were not present the following season. Perennial species were generally less affected than annuals. However, in Winsor Cove, high mortality and persistent impairment of growth were evident for annuals as well as for perennials. As found in Winsor Cove, certain species in Mill River continued to decline in abundance during the second season. Vegetation in Mill River in the "high marsh" and "mid-marsh" zones had substantially recovered by the 3rd and 4th yr, and the "low marsh" was unaffected. In Winsor Cove, however, the low marsh is still acutely affected and the less impacted high marsh has begun to recover in terms of average stem heights. In Winsor Cove, as previously mentioned, the mean stem number in both zones remained far below comparable numbers calculated from the control marsh.

These growth differences could be explained considering that the sediment of the lower intertidal zone was more impregnated with fuel oil due to the absorptive ability of the peat and its proximity to the tide range. Exposure to initial oiling was more frequent in the lower intertidal zone than in the upper slope which sometimes was beyond the reach of high tide. In addition, the substrate of the upper marsh was generally sandier and more susceptible to purging from tidal exchange.

In addition to the endemic species composition of a salt- vs. a freshwater flora, one of the major differences between Burk's study and Winsor Cove may have been the influence of tidal amplitude and mixing through wave action. In the marine intertidal zone, a wider vertical area between low and high water was vulnerable to direct exposure to oil whereas an oil spill on a pond or river is more restricted by the absence of tidal exchange.

Hershner (1977) conducted investigations at an experimental site in Whittaker Creek and a control site at Goat Point Creek, both in the lower Chesapeake Bay. Whittaker Creek was intentionally dosed twice a month with No. 2 fuel oil from November 1973 to August 1974. The marsh was monitored for general physical changes, net production, decay rates, physical characteristics of the grasses, and hydrocarbon content of *S. alterniflora* and related sediments. *Salicornia*, which was reduced 52.5% by the oiling, was reported to be the only plant species to show harmful effects during the dosing period. Sublethal effects delayed the production of new shoots and the fall set was suppressed almost entirely.

## Erosion

The rate of erosion observed in Winsor Cove can be correlated with the degeneration of the intertidal marsh root system acting as a binding mechanism. The similarity of sediments, flora, and the locations of both these marshes at the head of protected harbors made other interpretations unlikely. These data substantiate the findings of Teal and Teal (1969), and Wass and Wright (1969) who have found that marine marsh communities have the ability to bind loose sediments and serve in reducing erosion by dissipating wave and tidal energy.

Hershner (1977) found in Whittaker Creek that after the disappearance of a vigorous *Spartina* stand the topography of the creek was altered. Two previously vegetated spits eroded after defoliation and destruction of *Spartina* roots and rhizomes. The result was a loss of 8–10 cm from the vertical profile of the spits, and 50–100 cm in the horizontal profile.

## Microscopic Algae

The relative absence of blue-greens at Winsor Cove contrasts with 19 species reported from a healthy marsh in Great Britain (Stewart and Pugh 1963), over 20 species reported from a marsh at Ipswich, Massachusetts (Webber 1967), and 32 species in Delaware marshes (Ralph 1977). Therefore, even though the blue-greens have colonized the surface of the peat, the number of species present does not compare with data from healthy marshes studied.

A blue-green algal mat was not present at the Chappaquoit Marsh site where the substrate was sandy at the seaward edge. Microscopic algae were rare and scattered. *Lyngbya aestuarii* was the dominant blue-green species. Species of *Cladophora* and *Rhizoclonium* occurred in the first quadrat on several occasions. Due to the almost complete cover of *Spartina alterniflora* and *Salicornia* spp. shading the substrate, the abundance of surface algae was diminished. Ralph (1977) indicates that there is a correlation between the mean amount of light available at the soil surface and the total number of blue-green algae observed. The lack of vegetation allowing increased light penetration is probably a reason for the development of an algal mat at Winsor Cove. The less consolidated substrate may also be a contributing factor.

A review on the effects of oils and oil components on algae by O'Brien and Dixon (1976) relate several studies that show the relatively less damaging effects that weathered oil has on salt-marsh plants.

Studies by Hershner (1977) and Baker (1971) seem to confirm that marshes are able to withstand or recover rather quickly from a "single dose" of oil. However, Winsor Cove, which could be categorized as having received a "single dose" type oil spill, actually received frequent repetitive applications of oil as determined by tidal oscillations and wind direction. Harbors, rivers, and embayments are very susceptible to this repetitive type exposure.

During the initial spill in Winsor Cove, environmental

conditions were such that the oil collected into a protected cove and the slick remained for a few days. The substrate of the marsh, acting as a sponge, became heavily impregnated with oil due to the porosity of the marsh and interstitial absorption. Weathering of the impregnated oil was extremely limited in the absence of adequate oxygen and flushing. The slow chronic discharge of buried oil contained toxic aromatics that leached to the surface substrate causing a continuous stress on plant regeneration.

Other studies reporting oil impregnating sediments and long residence times show that the Bourne 1974 spill is not unique. Such results were evident in the West Falmouth oil spill of 1969 (Burns 1975; Blumer et al. 1971; Blumer and Sass 1972a, b; Sanders et al. 1977). Similarly, Mayo et al. (1975) reported fresh oil leaching from saturated sediments up to 18 mo following the initial detection of the spill in Long Cove, Searsport, Maine, involving JP5 jet fuel and No. 2 fuel oil. The Bunker C oil spill in Chedabucto Bay, Nova Scotia, documented by Vandermeulen and Gordon (1976) revealed a slow release of petroleum hydrocarbon from saturated sediments and rooted eelgrass for a minimum of 5 yr.

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### Appendix 1

Species list of dead and moribund invertebrates from qualitative samples from Bassett's Island and Winsor Cove.

| | Winsor Cove | | | Bassett's Island | | | | |
|---|---|---|---|---|---|---|---|---|
| | Oct. 14, 1974 | | | Oct. 12, 1974 | | | Oct. 13, 1974 | |
| Bourne oil spill | no. 1 | no. 2 | no. 3 | no. 1 | no. 2 | no. 3 | no. 4 | no. 5 |
| Polychaetes | | | | | | | | |
| *Amphitrite ornata* | | | | | | | 1 | |
| *Prionospio heterobranchia* | | | | | | | 1 | |
| *Sthenelais boa* | | | | 3 | 2 | | 27 | 3 |
| *Arabella iricolor* | | | | | | | 10 | |
| *Glycera americana* | | | | 5 | 6 | | 21 | 17 |
| *Pectinaria gouldii* | 1 | | | 1 | | | 2 | |

**Appendix 1 (Continued)**

| | Winsor Cove | | | Bassett's Island | | | | |
|---|---|---|---|---|---|---|---|---|
| | Oct. 14, 1974 | | | Oct. 12, 1974 | | | Oct. 13, 1974 | |
| Bourne oil spill | no. 1 | no. 2 | no. 3 | no. 1 | no. 2 | no. 3 | no. 4 | no. 5 |
| *Scoloplos fragilis* | | 1 | | 1 | | | 1 | |
| *Phyllodoce arenae* | | | | | | | | 1 |
| *Eumida sanguinea* | | | 1 | 3 | 2 | | | 6 |
| *Podarke obscura* | | | | | | | | 1 |
| *Pista palmata* | | | | | | | | 12 |
| *Goniada maculata* | | | | 6 | | | | |
| *Glycera dibranchiata* | | | | | 10 | | | |
| *Scoloplos robustus* | | | | | 1 | | | |
| *Scoloplos* (juv's) | | | | | 3 | | | |
| *Eteone* sp. | | | | | 2 | | | |
| *Eteone lactea* | | | | | 1 | | | |
| Syllid sp. | | | | | 1 | | | |
| Nereid sp. | | | | | 1 | | | |
| *Eusyllis lamelligera* | | | | | 1 | | | |
| *Sphaerosyllis hystrix* | | | | | 1 | | | |
| *Brania clavata* | | 11 | | | 1 | | | |
| *Arenicola cristata* | | | 1 | | | | | |
| *Exogone dispar* | | | 3 | | | | | |
| *Syllides verrilli* | | 2 | | | | | | |
| *Polydora ligni* | | 1 | | | | | | |
| Amphipods | | | | | | | | |
| *Gammarus mucronatus* | | | | 9 | | | | |
| *Elasmopus levis* | | | 1 | 9 | 84 | | 13 | |
| *Melita nitida* | | | | 8 | 5 | | 1 | |
| *Cymadusa compta* | | | | 15 | 1 | | 1 | |
| *Batea catharinensis* | | | | 3 | 2 | | | |
| *Corophium acutum* | | | | 3 | 2 | | | |
| *Lysianopsis alba* | | | 1 | 181 | 153 | | 98 | |
| *Ampelisca vadorum* | | | | 15 | 11 | | 30 | |
| *Listriella barnardi* | | | | 9 | 1 | | 3 | |
| *Paraphoxus spinosus* | | | | 151 | 256 | | 65 | |
| *Microprotopus raneyi* | | | | 2 | 1 | | 1 | |
| *Trichophoxus epistomus* | | | 1 | 4 | 5 | | 2 | |
| *Ampelisca verrilli* | | | | 1 | 1 | | | |
| *Hyale nilssoni* | | | | 1 | | | | |
| *Rudilemboides naglei* | | | 1 | 10 | 14 | | 199 | |
| *Microdeutopus gryllotalpa* | | | | 26 | 17 | | 11 | |
| Unidentif. Amphipod sp. | | | | 2 | | | | |
| *Lembos smithi* | | | | | 6 | | 4 | |
| *Jassa falcata* | | | | | 1 | | 1 | |
| *Ampithoe rubricata* | | | | | | | 2 | |
| *Talorchestia megalophthalma* | | | | | 2 | | | |
| *Caprella penantis* | | | | 26 | 1 | | | |
| *Monoculodes edwardsi* | | | | 1 | | | | |
| Decapods | | | | | | | | |
| *Upogebia affinis* | | | 3 | | 1 | | 5 | 1 |
| *Panopeus herbstii* | 2 | 1 | 1 | 30 | 30 | | 4 | 41 |
| *Pagurus longicarpus* | | 2 | 2 | 15 | 17 | | 7 | 17 |
| *Palaemonetes intermedius* | | | 1 | | 1 | | 4 | |
| *Callinectes sapidus* | | | | | | | 1 | |
| *Ovalipes ocellatus* | | | | | | | | 3 |
| *Carcinus maenas* | 2 | 7 | | 5 | 20 | | | 2 |
| *Emerita talpoida* | | | | | | | | 2 |
| *Sesarma reticulum* | 1 | | | | | | | |
| *Heteromysis formosa* | | | | 5 | 2 | | | 2 |
| *Palaemonetes pugio* | | | | | 1 | | | |
| *Uca pugnax* | 9 | 7 | | | | | | |
| Isopods | | | | | | | | |
| *Erichsonella filiformis* | | | | | | | 1 | |
| *Sphaeroma quadridentatum* | | | | 116 | 69 | | | 10 |

**Appendix 1 (Concluded)**

| Bourne oil spill | Winsor Cove | | | Bassett's Island | | | | |
|---|---|---|---|---|---|---|---|---|
| | Oct. 14, 1974 | | | Oct. 12, 1974 | | | Oct. 13, 1974 | |
| | no. 1 | no. 2 | no. 3 | no. 1 | no. 2 | no. 3 | no. 4 | no. 5 |
| *Gnathia cristata* | | | | | | | | 1 |
| *Idotea baltica* | | 7 | | | 4 | | | |
| Cumaceans | | | | | | | | |
| *Cyclaspis varians* | | | 1 | 177 | 44 | | 6 | 74 |
| *Oxyurostylis smithi* | | | | 2 | 3 | | | 2 |
| Tanaids | | | | | | | | |
| *Leptochelia savignyi* | | 3 | 5 | 45 | 35 | | 8 | 101 |
| Copepods (Harpacticoids) | | | | 115 | 90 | | 20 | 75 |
| Copepods (Cyclopoid) | | | | 5 | 9 | | | 6 |
| Ostracods | | | | 83 | 80 | | | 84 |
| Holothurians | | | | | | | | |
| *Thyone biriaeus* | | 2 | 8 | | | | 8 | 1 |
| *Cucumaria pulcherrima* | | | | | | | 3 | |
| *Leptosynapta tenuis* | | | 6 | 6 | 6 | 1 | 8 | 1 |
| Nemertine sp. A. | | | | | | | 3 | 5 |
| Sipunculids | | | | | | | | |
| *Goldfingia gouldi* | | | | | 5 | 2 | | 1 |
| Sipunculid sp. | | | | | 1 | | | |
| Fishes | | | | | | | | |
| *Gobiosoma hosei* | | | | | | | 1 | |
| *Tautogolabrus adspersus* | | | | 1 | | | | |
| Pelecypods | | | | | | | | |
| *Laevicardium mortoni* | | | 7 | 43 | 14 | | 11 | 3 |
| *Ensis directus* | | 2 | | 3 | 4 | 4 | | 3 |
| *Macoma tenta* | | 2 | 2 | 19 | | | | 3 |
| *Tellina agilis* | | | | | 6 | | | 3 |
| *Tagelus plebeius* | | | | 1 | | | | |
| *Venus mercenaria* | | | | 4 | 3 | | | |
| *Mya arenaria* | 1 | | | | | | | |
| *Pecten irradians* | | 1 | | | | | | |
| *Gemma gemma* | | 1 | 1 | | | | | |
| *Macoma balthica* | | | 1 | | | | | |
| *Cumingia tellinoides* | | | 1 | | | | | |
| Juvenile bivalve no. 5 | | | | 2 | | | | |
| Gastropods | | | | | | | | |
| *Urosalpinx cinerea* | | 10 | | 1 | 1 | | 5 | |
| *Littorina littorea* | | 2 | 1 | | | | 2 | |
| *Crepidula fornicata* | | | | | | | 3 | 2 |
| *C. convexa* | | 1 | 5 | 402 | 120 | | 3 | 98 |
| *Busycon canaliculatum* | | | | | | | | 3 |
| *Turbonilla?* | | | | | 2 | | 1 | |
| *Bittium alternatum* | | 5 | 2 | 28 | 18 | | 5 | 2 |
| *Nassarius vibex* | | | | | 1 | | | |
| *Caecum pulchellum* | | | | | 2 | | | |
| *Littorina obtusata* | | 62 | | | | | | |
| *Rictaxis punctostriatus* | | 8 | | | | | | |
| *Nassarius obsoletus* | | 1 | | | | | | |
| *Odostomia bisuturalis* | | 2 | | | | | | |
| *Turbonilla interrupta* | | 1 | | | | | | |
| *Littorina saxatilis* | | 147 | | | | | | |
| *Caecum cooperi* | | | 1 | | | | | |
| *Mitrella lunata* | | | 1 | | | | | |
| Amphineura | | | | | | | | |
| *Chaetopleura apiculata* | | | 1 | | | | 1 | |

# Long-Term Effects of an Oil Spill on *Fucus* Macrofauna in a Small Baltic Bay[1]

MATS NOTINI

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NOTINI, M. 1978. Long-term effects of an oil spill on *Fucus* macrofauna in a small Baltic bay. J. Fish. Res. Board Can. 35: 745–753.

On October 6, 1970, the small tanker *Irini* ran aground in the southern part of the Stockholm archipelago, releasing about 1000 t of medium and heavy fuel oil. Approximately 400 t drifted into a small bay, Gästviken, wiping out nearly the entire littoral fauna. Most of the oil was collected mechanically during the winter, and by May 1971 cleanup operations were completed. The recruitment of the bladder wrack (*Fucus vesiculosus*) community in the bay was observed at intervals over a 5-yr period. Significantly increased macrofauna population densities were found for a number of species in the 1974 and 1976 samples compared to those of 1971 and 1972. From June–July 1971 to June 1976, the mean numbers of individuals for all species rose from about 280 to 1000/100 g *Fucus* dry weight (d.w.). The bivalve *Mytilus edulis* increased in number from 0 to about 45, the gastropod *Theodoxus fluviatilis* from 0 to about 160, the amphipod *Gammarus* spp. from about 40 to 580, the isopods *Idotea* spp. from about 5 to 35, and *Iaera* spp. from almost 0 to about 10/100 g *Fucus*. Larvae of Chironomidae were the only group found with a decreased density between the first and sixth summers after the spill, with 240 and 145 individuals, respectively. The data obtained are discussed in relation to conditions in a nearby unpolluted bay and to normally occurring cyclic variations.

*Key words:* algae, *Fucus vesiculosus*, littoral community, petroleum, pollution

NOTINI, M. 1978. Long-term effects of an oil spill on *Fucus* macrofauna in a small Baltic bay. J. Fish. Res. Board Can. 35: 745–753.

Le 6 octobre 1970 un petit pétrolier, l'*Irini*, échouait dans la partie méridionale de l'archipel de Stockholm, déversant environ 1 000 tonnes de fuel-oil moyen et lourd. Environ 400 tonnes dérivèrent dans une petite baie, la baie de Gästviken, anéantissant presque toute la faune littorale. La plus grande partie du pétrole fut enlevée mécaniquement au cours de l'hiver et en mai 1971, le nettoyage était terminé. Nous avons observé périodiquement, sur une période de 5 ans, le recrutement de la communauté associée aux raisins de mer (*Fucus vesiculosus*) dans la baie. Nous avons constaté pour plusieurs espèces une augmentation significative des densités de population de la macrofaune dans des échantillons prélevés en 1974 et 1976, comparativement aux densités de 1971 et de 1972. De juin–juillet 1971 à juin 1976, le nombre moyen d'individus de toutes les espèces passa de 280 à 1 000/100 g de *Fucus* (poids sec). Le nombre passa de 0 à environ 45/100 g de *Fucus* pour le bivalve *Mytilus edulis*, de 0 à environ 160 pour le gastropode *Theodoxus fluviatilis*, d'environ 40 à 580 pour l'amphipode *Gammarus* spp., d'environ 5 à 35 pour les isopodes *Idotea* spp. et de presque 0 à environ 10 pour *Iaera* spp. Les larves de Chironomidae furent le seul groupe où l'on a constaté une diminution de densité entre le premier et le sixième été qui suivirent le déversement, 240 et 145 individus respectivement. Nous analysons les données par rapport aux conditions observées dans une baie non polluée avoisinante et aux variations cycliques normales.

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ALTHOUGH oil spills frequently occur in the Baltic Sea, in situ studies of the effects are scarce. The only papers available are from the studies of the *Palva* oil tanker disaster in May 1969 in the Finnish southwest archipelago (Pelkonen and Tulkki 1972; Ravanko 1972). There are no reports on long-term observations of the effects of oil spills on fauna or flora in the Baltic Sea. About 200 oil spills are reported yearly in Swedish waters alone (Landner and Hagström 1975). The potential risks of a major oil spill in the Baltic ecosystem as a result of increasing traffic and tonnage are obvious.

[1]This paper forms part of the Proceedings of the Symposium on "Recovery Potential of Oiled Marine Northern Environments" held at Halifax, Nova Scotia, October 10–14, 1977.

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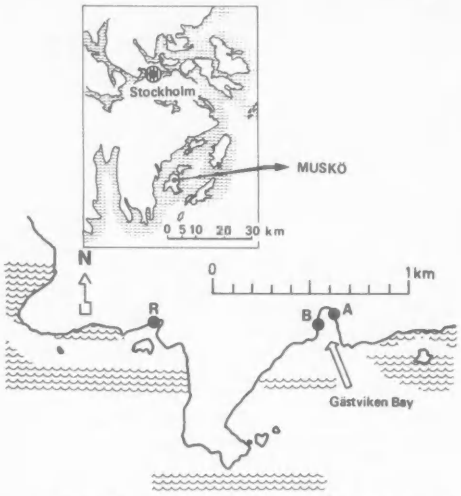


FIG. 1. Location of Muskö Island and stations used. Stations A and B are situated in oil polluted Gästviken Bay and station R in a bay used for reference.

Luckily, very few accidents involving more than 500 t have occurred so far. The largest known is the grounding of the oil tanker *Jawachta* on December 20, 1973 near the Baltic sill, when 2500 t of Venezuelan crude were released. This accident, however, resulted in only minor damage (Notini 1976).

In the shallow Baltic Sea with an extensive archipelago the littoral zone is of major importance. Acute and long-term effects from a major oil spill in this zone can be expected because a shore is never far distant. The dominating weed in large parts of the Baltic is the brown alga *Fucus vesiculosus* L. It has a maximum density at a depth of between 0.5 and 2 m which is, because of the great variety of niches, probably the most diverse and productive habitat in the Baltic shallow waters (Jansson and Wulff 1977).

On the foggy morning of October 6, 1970, the Greek tanker *Irini* (13 923 d.w.) carrying 5300 metric t of medium fuel oil (No. 3) and 1700 t of heavy fuel oil (No. 5) ran aground in the archipelago about 40 km south of Stockholm. Several tanks were broken by the impact and during the following 5 d approximately 1000 t of mostly medium fuel oil escaped into the sea. The Swedish coast guard used booms, skimmers, and detergents (Corexit 76 74, Finasol SC) to protect the shores from the spills. During the first 2 d a small bay, Gästviken, on the island Muskö, 4 km north of the grounding, was heavily polluted. Approximately 400 t of the oil forced by strong winds reached the bay. The wind later turned westward and the spills flowed in a more easterly direction. The coast guard managed to enclose the oil by laying booms across the bay. Most of the oil was then collected mechanically during November–December 1970. The final cleanup was done after the ice was gone. The oily sand was removed and rocks and stones were cleaned. Solvents were used to a limited extent. By the middle of May 1971, all cleaning in the bay was finished.

Gästviken is only 200 m long and 150 m wide at the mouth. It has a sandy beach with rocks and stones on both sides and a gradually decreasing depth from about 12 m. The bay is exposed to winds from the south and southeast.

The recruitment of the littoral community following the spills and the cleanup operations in the area have been observed from June 1971 to June 1976. Some of the results from the first 2 yr have been previously reported (Notini and Hagström 1974).

The present paper is a more detailed presentation of the observations made during the first 2 yr following the spill, together with continuous observations during 1974 and 1976.

## Materials and Methods

### *Fucus* COMMUNITY

Quantitative samples of the *Fucus* belt macrofauna were taken at three stations on the island Muskö, (Fig. 1). Two stations, A and B, are located close to one another, in the oil polluted Gästviken Bay. The third station, R, chosen as a reference, is located in a bay with no sign of oil pollution and is situated approximately 1 km west of Gästviken. All samples were taken by scuba diving at a depth of 1–1.5 m. Plastic bags were quickly placed over a *Fucus* plant randomly chosen from above. The seaweeds were then cut off by the diver close to the basal plates. To minimize the loss of animals due to mortality and predation, the samples were quickly transported to the laboratory for sorting and examination. All macrofauna specimens were picked out and preserved for later determination. Unfortunately, this procedure takes between 3 and 8 h/sample. The condition and relative amount of epiphytic algae of the *Fucus* plants were noted before the wet and dry weights of the algal samples were determined. In all, 50 samples were taken, with an average sample size of 108 ± 5 g d.w. (mean ± SE) *Fucus*. During 1971 and 1972 the number of samples taken at each station was three or less, before a more efficient procedure was introduced. In all sampling after 1972 the whole plastic bag with its content of algae, animals, and some water was quickly frozen. Later, the samples were thawed individually overnight prior to analysis. As a result the number of samples taken at each locality during 1974 and 1976 was increased to four.

All animals were counted and classified into systematic groups, in most cases to genera and species. Arithmetic means of density of the most abundant groups are given, whenever possible with its 95% confidence interval. For presentation purposes, the samples taken at stations A and B have been lumped, thus representing the whole Gästviken Bay in Fig. 3 and 4.

The degree of similarity between samples from different localities on different occasions was calculated using affinity indices. Usually such indices are computed from material determined to species but in the present work this was not always possible and higher order groups were sometimes used.

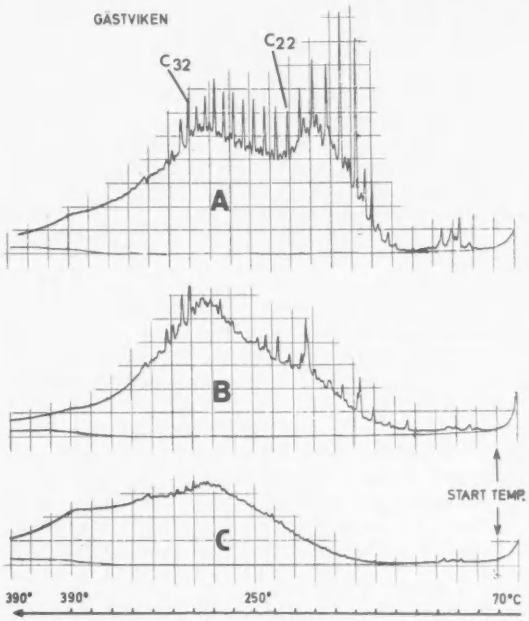


FIG. 2. Degradation of the oil in Gästviken during a 4-yr period. (A) Weathered oil slick, June 1971; (B) sediment from the bay, June 1975 (0–3 cm, depth 6 m); (C) oil on the shore, June 1975.

Two types of affinity indices were used: the coefficient of community and the percentage similarity of community. The coefficient of community ($CC$) is defined as

$$\frac{c}{a+b-c}100$$

where $a$ is the number of species in the first sample, $b$ is the number of species in the second sample, and $c$ is the number of species occurring in both samples. The number of individuals of each species is not taken into consideration in this index. This is done, however, in the index of percent similarity of community ($PS_c$), which is defined as

$$100 - 0.5\,\Sigma(a' - b');\ \text{or as}\ \Sigma\ \min\ (a', b')$$

in which $a'$ and $b'$ are for each species the respective percentages of the total number of individuals in samples A and B. The indices are discussed by Whittaker (1952) and Whittaker and Fairbanks (1958). The affinity indices for a pair of sample comparisons are further classified into groups of similar samples according to a method given by Mountford (1962).

### Oil Analyses

Qualitative gas-chromatographic analyses of oil samples from the shores and sediment in Gästviken Bay were performed using a Varian 1400 FID. Hexane extracts fractionated by passing through a potassium hydroxide column were injected into a glass column (150 × 0.2 cm) containing 3% Dexsil 300 on Varaport 30 (100–120 mesh). The injection and detection temperatures were 370°C, the column temperature programed at 20°C/min from 70 to 390°C, and the carrier gas was $N_2$ (30 mL/min).

## Results

### Fate of Oil

On the first visit to Gästviken in June 1971, remarkably few signs of the massive oil spill or the newly finished cleanup operations were found. Embedded in sand on the beach and below some of the stones on the shore, oil films and slicks were found, but below the water surface no visible oil was observed. However, sediment analyses proved different.

In Fig. 2 the fate of oil after a 4-yr period in the sediment and on the shore of the bay is illustrated. The extract from a weathered oil slick (A) found in June 1971 is compared to extracts from oil on the shore (C) and from an oil contaminated sediment (B) during sampling in June 1975. As shown by the gas chromatograms, the rate of degradation of oil on the shore is much faster than in the sediment. In the sediment the amount of n-alkanes with a chain length less than $C_{32}$ is reduced and the original $C_{15}$-$C_{22}$ n-hydrocarbons have disappeared, probably due to biodegradation.

### Macrophytic Algae

No effects on macrophytic algal density and succession have been observed in the bay other than those that can be explained by natural ecological forces. During the first summer and autumn of 1971, the normal zonations and shifts of species in the area seem to have occurred. The green alga *Cladophora glomerata* (L.) Kütz. commonly covered stones and rocks down to about 0.5 m during the summer. Other green algae, among them *Enteromorpha* spp., were also abundant. In November, the red alga *Ceramium tenuicorne* (Kütz.) frequently appeared from 0.3 to 1.5 m. The quantitatively most important alga in the bay was the brown alga *Fucus vesiculosus* L., which was found in the sublittoral zone from 0.5 to 2 m on stones and rocks along both sides of the bay. During June–July few epiphytic algae were found on the *Fucus*, but later in the summer they were common. On the sandy bottom in the middle of the bay submerged phanerogams dominated. *Zostera marina* L. commonly occurred from 2 to 5 m and *Potamogeton pectinatus* L. and *P. filiformis* Pers. also appeared in irregular patterns. All three species were green and growing well during the summer.

### *Fucus* Macrofauna

*Seasonal variations* — From June to November 1971 following the oil spill, 24 animal species or groups were found in the *Fucus* "bag" samples from Gästviken Bay (Table 1). From only 5 groups in June, the number increased to 10, 16, and 14 in July, August, and November, respectively. A similar composition was found in the two samples from the reference bay in

TABLE 1. Species composition of *Fucus* macrofauna in samples from Gästviken Bay 1971, the first year following the oil spill. Present species are marked by +.

| | June | July | Aug. | Nov. |
|---|---|---|---|---|
| Bryozoa | | | | |
| *Membranipora crustulenta* Pallas | | | | + |
| Platyhelminthes spp. | | | | + |
| Oligochaeta spp. | | + | | |
| Hirudinea | | | | |
| *Piscicola geometra* L. | | | + | + |
| Crustacea | | | | |
| *Balanus improvisus* Darwin | | | | + |
| *Neomysis integer* Leach | | | + | |
| *Leander adspersus* Rathke | | + | + | |
| *Gammarus oceanicus* Segerstråle | + | + | + | + |
| *G. zaddachi* Spooner | + | + | + | + |
| *G. salinus* Spooner | | | + | |
| *G. locusta* Spooner | | | + | |
| *Iaera albifrons* group Leach | | + | + | |
| *Idotea baltica* Pallas | + | + | + | + |
| *I. chelipes* Pallas | | | + | |
| Mollusca | | | | |
| *Mytilus edulis* L. | | | | + |
| *Cardium* spp. | + | + | | + |
| *Theodoxus fluviatilis* L. | | + | + | + |
| *Lymnea* spp. | | + | + | + |
| *Hydrobia* spp. | | | + | + |
| Chironomidae | | | | |
| *Orthocladius* + *Cristopus* spp. | + | + | + | |
| Pisces | | | | |
| *Gobius niger* L. | | | + | |
| *Pomatoschistus minutus* Pallas | | | + | |
| *Gasterosteus aculeatus* L. | | | | + |
| *Pygosteus pungitius* L. | | | | + |

July and August with the exception of some additional trichopterous larvae. In Fig. 3, the seasonal variation in density of five dominant groups together with the total number of individuals in all species are quantified per 100 g *Fucus* d.w. Between June, July, and August the number of the crustaceans *Gammarus* spp. and *Idotea* spp. increased significantly in Gästviken, from about 40 to 600 and from 2 to 50, respectively. A parallel increase in single samples from the reference bay was also found but the density was much higher (about 330 to 1070 and 25 to 200, respectively). In November 1971, the *Gammarus* spp. population in Gästviken dropped to a level similar to that in June–July, a little over 30 per 100 g *Fucus*, while the *Idotea* spp. population density was still significantly increased. The populations of gastropods *Lymnea* spp. and *Theodoxus fluviatilis* slowly increased from almost 0 in June–July to 20 and 8, respectively, in November.

In the reference bay, the *Lymnea* spp. population seemed to increase faster than in Gästviken (from 3 to 50 in the two samples taken), *Theodoxus fluviatilis* specimens, on the other hand, seemed stable at about 180, i.e. about 9 times higher than in Gästviken. The chironomid larvae population significantly decreased in Gästviken between June–July and August 1971, from about 260 to 6, while the population in the reference bay was low, about 10 in both samples. The overall curve for animal specimens found in Gästviken in 1971 increased from about 310 in June–July to 690 in August, followed by a decrease to 235 in November. The values from the single samples in the reference bay were almost twice as high both in July and in August, about 595 and 1530 individuals per 100 g *Fucus* d.w., respectively.

*Annual variations* — The density figures from 1971 and the overall picture indicate rapid recruitment in the area. Continuous sampling of *Fucus* macrofauna in Gästviken, however, gave a somewhat different picture. In Fig. 4, the arithmetic means for the same dominant animal groups added to the bivalves *Mytilus edulis* and *Cardium* spp. are given, from June–July 1971, and June 1972, 1974, 1976, together with values from the reference bay for June 1974 and 1976. There were, unfortunately, no samples taken in 1972 in the reference bay. In a number of comparisons the means from 1971 and 1972 significantly ($P < 0.05$) differ from the 1974 and 1976 year means as indicated by the non-overlapping confidence intervals. The amphipod *Gammarus* spp., both adults and juveniles ($\leq$10 mm), had significantly increased from June–July 1971 and June 1972 to June 1976 (4, 9, and 210, respectively, 37, 44, and 370 individuals per 100 g *Fucus* d.w.). The total gammarid population in the reference bay is significantly lower in 1974 and 1976 compared to Gästviken, while the single sample taken in July 1971 has populations 6 times higher than in Gästviken during the same period (Fig. 3). The isopods *Iaera* spp. and *Idotea* spp. significantly increased from the first to the 6th yr following the accident. The bivalve *Mytilus edulis* was completely missing in the *Fucus* samples during the first three sampling periods in Gästviken during 1971. In July 1972 the mean in six samples was 4. In July 1974 and 1976 the *Mytilis* population density is markedly higher, 52 and 45 per 100 g *Fucus* while at the same time noticeable fluctuations in the reference bay samples occurred. The total number of *Mytilus edulis* specimens (783) found in Gästviken "bag" samples ($n = 32$; total *Fucus* d.w. 3.7 kg) have been classified into different shell-length groups (Fig. 5). During the first 2 yr following the spill, only a few larger and newly settled specimens were found on the two localities in the bay.

The settling of new larvae in the inner part of the bay (station A, Fig. 1) was probably disturbed. In 1974 and 1976 a more normal length distribution occurred in the populations. The bivalve *Cardium* spp. population (Fig. 4) in Gästviken was low, about 2–3 individuals per 100 g *Fucus* during 1971, 1974, and 1976. In 1972 no *Cardium* spp. were found in the samples. The density of *Cardium* spp. in the reference bay in 1974 and 1976 is significantly higher (31 and 7

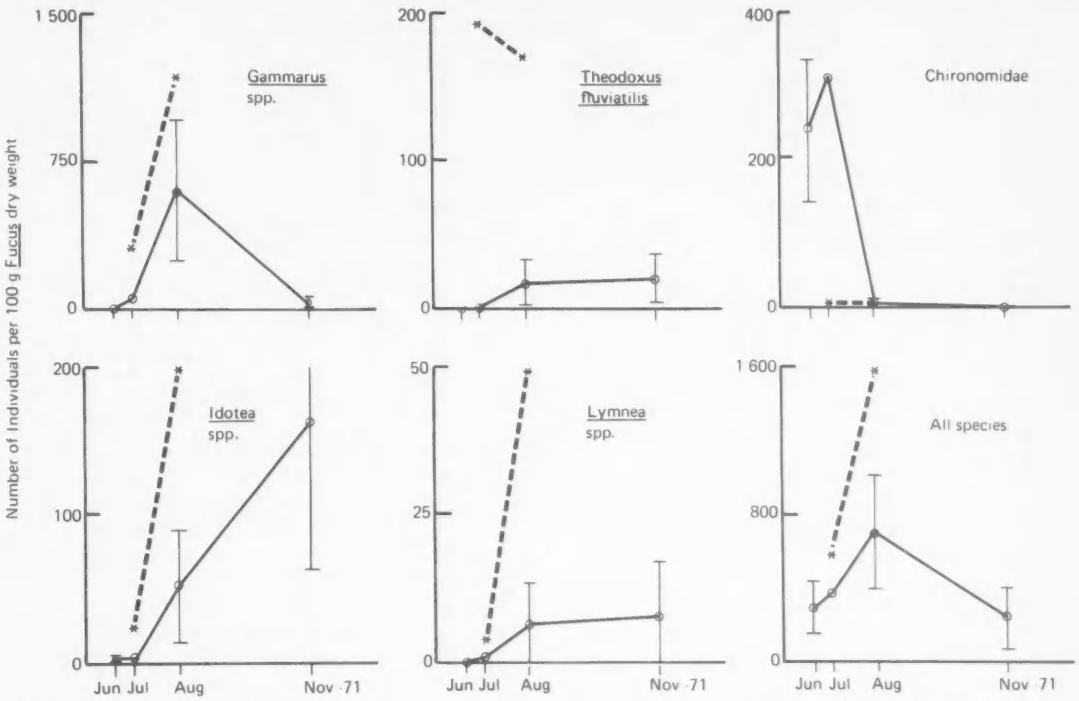


FIG. 3. Number of macrofauna specimens in *Fucus* samples during the 1st yr, 1971, following the oil spill. * – – – – * reference bay, ○ ——— ○ Gästviken, ⌶ = 95% confidence interval for population mean.

individuals per 100 g *Fucus* d.w.). The mean number of gastropod *Lymnea* spp. found in the Gästviken Bay is lower in 1974 and 1976 than in the reference bay. Another gastropod, *Theodoxus fluviatilis*, markedly increased from 0 and 18 in 1971 and 1972, respectively, to 335 and 162/100 g *Fucus* in 1974 and 1976. The only animal group with a significantly higher mean during the 1st yr, compared to the following years in the oil polluted bay are Chironomidae spp. larvae, but this group shows great normal cyclic variations as illustrated by data from the reference bay. In spite of decreasing Chironomidae populations, the overall picture of total animal species (Fig. 4) shows a significant increase in population means. The total number of individuals found in Gästviken in 1971 and 1972 are 3.5–8 times lower than the means in 1974 and 1976, (about 275 and 125, respectively, 980 and 1010 per 100 g *Fucus* d.w.).

*Community structure* — The degree of similarity of the *Fucus* macrofauna community at the three localities A, B, and R is illustrated in the dendrograms (Fig. 6).

Two types of affinity indices $PS_c$ and *CC* used on sample means from the bays are classified according to the Mountford (1962) method. As can be seen in the dendrograms, the *Fucus* fauna composition at both localities A and B in Gästviken in June 1971 are very similar but have very little in common with any other sample means in the reference bay or in Gästviken in the following years. Another conclusion from the dendrograms is that species composition is similar in the two bays but the percentage similarity of abundant species differs.

## Discussion

Seasonal cyclic variations in the littoral fauna and flora, as well as yearly variations, occur by operation of natural ecological forces. Variations in water temperature together with ice cover and strong winds are probably the most important environmental factors causing these fluctuations in the area studied. The water temperature and winds of the area were taken into account when deciding sampling dates. Kautsky (1974) showed a decrease in the macrofaunal population in the sublittoral zone following a storm. Storms normally occur in the area during autumn or winter, mostly from the NW and SW. During May and June 1971–76 only one storm occurred, June 13–14, 1973. In Fig. 7 the average surface water temperatures (°C) for each month during 1970–76 from this part of the Stockholm archipelago are given based on weekly data collected at a station approximately 30 km northeast of Gästviken Bay. The fluctuations in surface water tem-

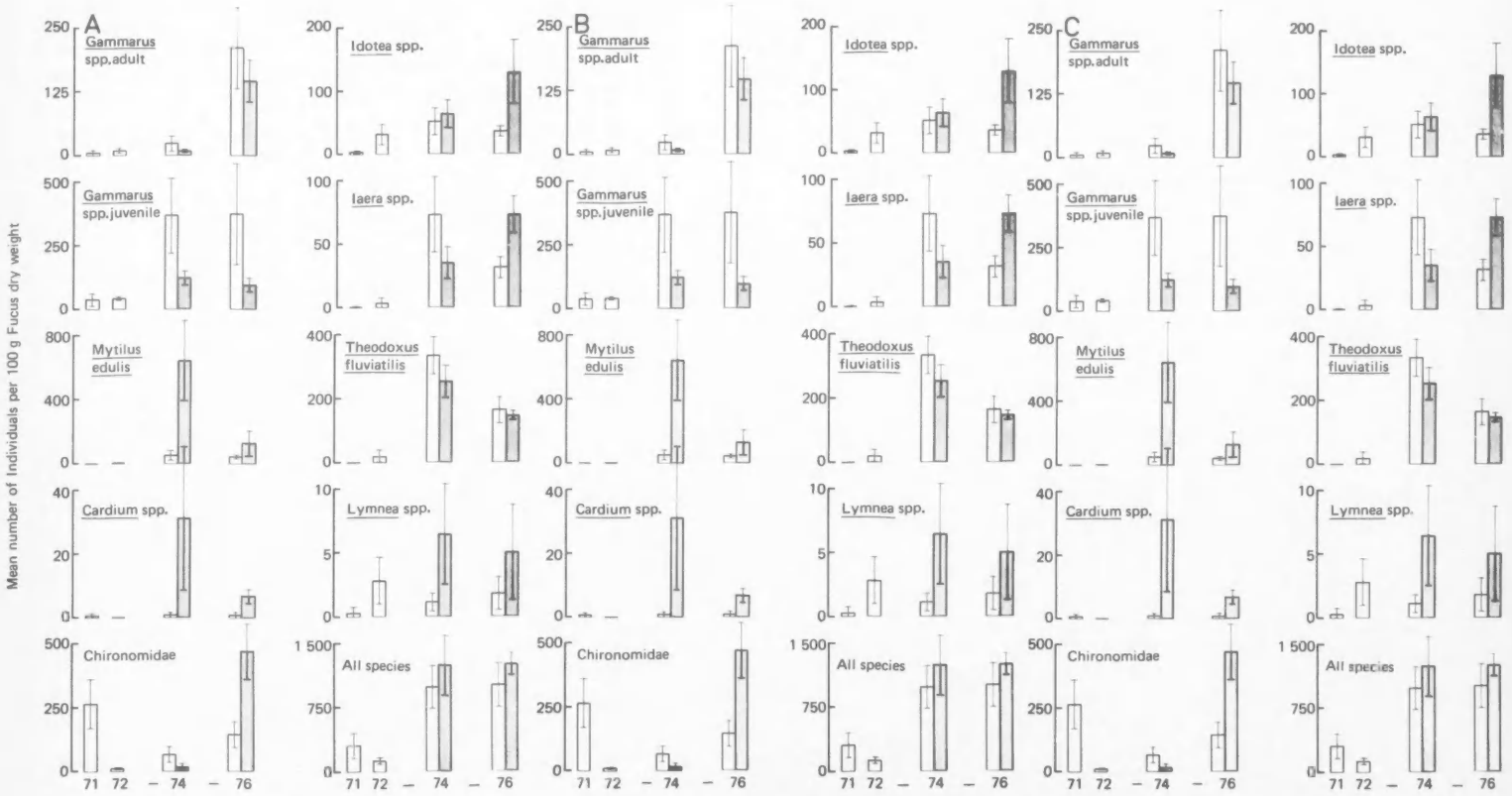


FIG. 4. Number of macrofauna specimens in *Fucus* samples. Sample size: Gästviken June–July 1971 $n = 3$, June 1972, 1974, and 1976 $n = 6$, 8, and 8. Reference bay June 1974 and 1976 $n = 4$. White bars = Gästviken Bay; shaded bars = reference bay; $\perp$ = 95% confidence interval for population mean.

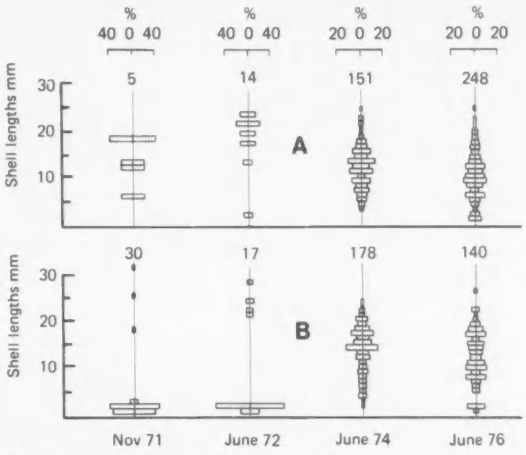


FIG. 5. Size distribution (%) of *Mytilus edulis* specimens found in *Fucus* samples at stations A and B in Gästviken. Total number of specimens is given above each column. During June–August 1971 no specimens were found.

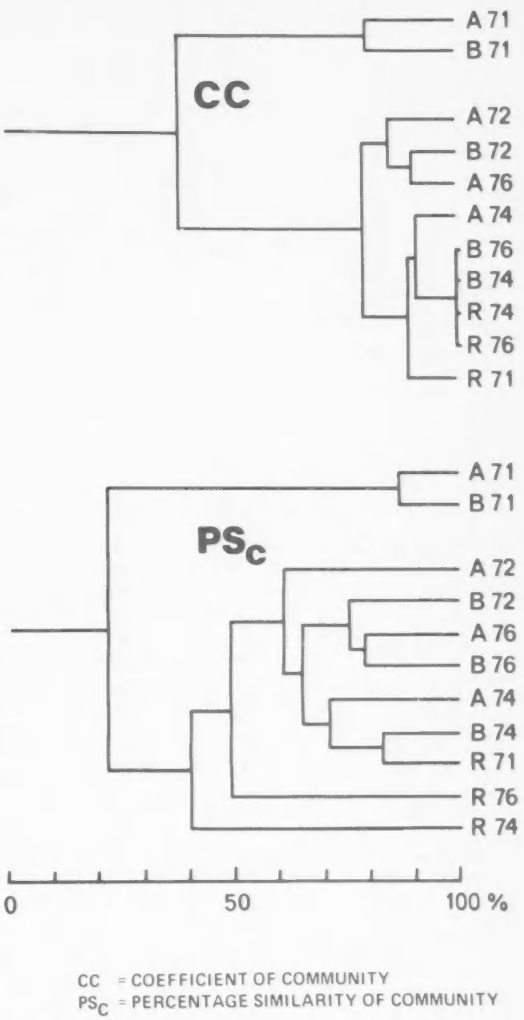


FIG. 6. Dendrograms illustrating classification of the samples and the values of indices of similarity between samples. A, B, and R are the sampling locations as in Fig. 1.

perature in the area are great. The normal amplitude within a year is about 15°C. The winters 1969–70 and 1970–71 were cold, the winters 1972–73 and 1974–75 unusually warm, and the winters 1971–72, 1973–74, and 1975–76 were normal. The arrow 1 indicates the date of the grounding and 2 the end of the cleanup operations. Arrows 3 and 9 indicate when samples were taken in the oil polluted bay. Unfortunately, this temperature curve correlates with some of the population density figures found in Gästviken. After the cold winter in 1970–71, with ice in the area, lower density could be expected for many of the littoral and sublittoral species, because the ice cover influences the organisms in many ways, often indirectly. The organisms are affected mechanically, especially during the spring breakup of the ice. The algae are often affected by the attenuation of light by the ice cover, particularly when snow-covered, and ice cover also reduces the turbulence of the water, its oxygen content, salinity, and nutrient supply (Wallentinus 1976).

*Fucus vesiculosus* has proved resistant to light and heavy fuel oil exposure in outdoor laboratory tests (Ganning and Billing 1974) and the mucilage layer of the alga may prevent adhesion of the oil (Stebbings 1970). Thus, no irreversible injury to the perennial *Fucus* plants occurred during the first winter.

In July 1971 the annual algal crop was low, probably due to both the cold winter and the oil, but since most of the oil was removed before the beginning of the vegetative period in the middle of May, the algal community in the bay did not differ from that normally found in the area. Ravanko (1972), observed no adverse effects on aquatic flora from the *Palva* oil spill, apparently due to the fact that the spill had occurred before the annual spring algal growth. An oil spill in summer might have had greater effects. In an outdoor model littoral ecosystem (Notini et al. 1977), simulated oil spills during the summer have resulted in markedly increased green and blue-green alga stocks, (M. Notini unpublished data).

The total number of animals collected per 100 g *Fucus* d.w. in a sample from Gästviken varied from 104 at station A in November 1971 to 1612 at station B in June 1974. These figures are in agreement with those found by Haage (1975), using similar methods at different seasons in the unpolluted Askö area (about 30 km SE of Muskö Island).

There are, of course, no identical bays with regard

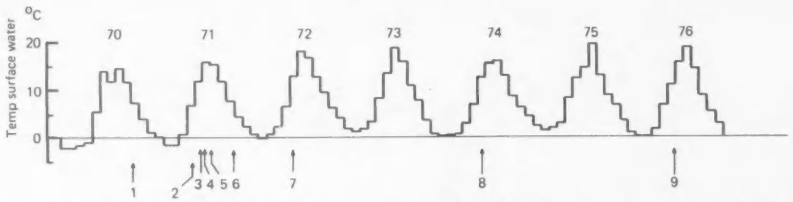


FIG. 7. Temperature (°C) of surface water 1970–76. (Monthly mean of data provided by Stockholm City Street and Traffic Department.) Arrow (1): *Irini* accident October 6, 1970, (2): end of cleanup May 16, 1971, (3–9): sampling dates, (3) June 24, 1971, (4) July 7, 1971, (5) August 8, 1971, (6), November 2, 1971, (7) June 1, 1972, (8) June 17, 1974, (9) June 29, 1976.

to faunal and floral comparison. Gästviken is slightly more exposed from the south than the "reference" bay, which is sheltered by an island (Fig. 1), but there are no other more suitable bays in the area. Haage (1975) found that the most striking differences between sheltered and exposed *Fucus* communities were the absence of insects and certain *Lymneidae* and the lower densities of *Cardium* spp. in the latter. This is probably the reason why higher densities of the *Lymnea* spp. and *Cardium* spp. (Fig. 4) populations occurred in the reference bay compared to Gästviken on the last two sampling occasions.

While recolonization of most species occurred in Gästviken during the 1st yr following the spill (Table 1, Fig. 3), unusually low population densities in the bay during 2 yr indicate intensive disturbance of the littoral fauna.

In the study of the *Palva* oil spill, Pelkonen and Tulkki (1972) found no clear deleterious effects on the littoral fauna when sampling in the polluted area 3 mo and 1 yr after the accident. They used nonquantitative methods, and only subjective estimates could be drawn. One of these was that larvae of the *Chironomus plumosus* group were numerous on dead *Fucus* during the first sampling (August 1969) but absent during the second (August 1970). Leppäkoski and Lindström (1978) and Bengtsson and Berggren (1972) have found that *Chironomus plumosus* larvae are sensitive to oil contaminated sediments. Other chironomid larvae with different habitats may, however, respond completely differently to oil pollution as the numerous *Orthochadinae* spp. found on the *Fucus* samples from Gästviken. In the pool experiments these groups were found to be one of few insensitive to oil pollution (M. Notini unpublished data). One of the other observations made by Pelkonen and Tulkki (1972), was that some crustacean species were absent at certain of the polluted localities after 3 mo, but occurred commonly after a year.

Susceptibility of various crustaceans to oil is known to differ with respect to the mobility, age, habitat, body surface etc. of the particular species. Most crustaceans have a cuticle covered with a hydrophobic, i.e. a lipophilic wax layer (Richards 1951). Species with strongly hydrophobic body surfaces such as *Gammarus* spp. accumulate hydrophobic substances (Nagell et al. 1974). Direct contact with oil for such species is therefore most often lethal; this has been shown in simulated oil spills in a littoral pool ecosystem (Notini and Hagström 1974). They found that with light fuel oil and Nigerian crude oil, *Gammarus* spp. were totally eliminated, while a few individuals survived a medium fuel spill during a 2-mo observation period. The survival might be explained by a diminished (acute) toxicity combined with a decreased stickiness.

Recolonization in an oil contaminated, geographically restricted locality with unpolluted surroundings such as Gästviken is to a great extent dependent on the mobility of the organisms as well as circulation of the water resulting in a reduction in the concentration of the remaining oil.

This can be seen in Table 1 and Fig. 3. Highly mobile organisms, such as insects, fish, and swimming crustaceans seem to recolonize the bay faster than animals with lower mobility. This may explain the abundance of chironomid larvae, and the relatively faster increase of *Gammarus* spp. and *Idotea* spp. during the first summer after the oil spill, compared, for instance, with the molluscs. The rather high number of *Gammarus* juveniles in 1971 and 1972 indicates that the toxicity of the remaining oil must be very low in the water. Lindén (1976) found that larvae of *Gammarus oceanicus* from the Baltic Sea were several hundred times more sensitive to acute oil exposure than adults.

It has already been mentioned that no signs of oil on or under the water surface — except in the sediments — were found. This, coupled with the fast renewal of the water in the bay, explains the obviously low toxicity.

However, it cannot be concluded that reproductive disturbances in the polluted area might not have occurred. Even if the level of toxicity is not lethal, the possibility cannot be excluded that some oil components leaching from the contaminated sediments have a repressive influence on the recruitment of the organisms. This may explain the weak settlement of *Mytilus edulis* larvae in the inner part of the bay (see Fig. 5) as well as the significantly lower appearance of *Gammarus* spp. in 1971 and 1972. Percy (1977) has found this effect in the case of a benthic amphipod.

The investigation in Gästviken shows that the re-

covery ability of the littoral community is good under the given conditions, i.e. intensive, mainly mechanical, cleaning, rapid water exchange and a geographically restricted polluted locality. In spite of the initially heavy degree of pollution and the resulting almost total faunal mortality, considerable recolonization took place during the first summer after the oil spill. After 4 yr, no significant evidence of lasting detrimental effects can be found when natural annual variations in the ecological system are taken into account.

## Acknowledgments

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# Observations of a Cold-Water Intertidal Community After 5 Years of a Low-Level, Persistent Oil Spill from the *General M.C. Meigs*[1]

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A rich and productive intertidal community was exposed continually for over 5 yr to small quantities of a Navy Special fuel oil from the unmanned troopship *General M.C. Meigs* that came aground on the Washington coast in January 1972. Observations of animal and plant populations and their petroleum hydrocarbon uptake patterns showed early evidence of contamination and the persistence of the oil spill throughout the study period. Abnormal and dead urchins, and loss of algal fronds and pigment were observed in localized areas near the wreck for at least 1 yr. Within 2 mo of the accident, paraffinic hydrocarbons had been taken up by prominent members of the community and continued to appear in certain species even after 5 yr. Although changes were seen in certain species during the early days of this persistent low-level pollution incident, the community balance in this rocky, intertidal ecosystem does not appear to have been markedly altered.

*Key words:* oil spill, hydrocarbons, *General M.C. Meigs*, petroleum contamination, biological effects

CLARK, R. C. JR., B. G. PATTEN, AND E. E. DENIKE. 1978. Observations of a cold-water intertidal community after 5 years of a low-level, persistent oil spill from the *General M.C. Meigs*. J. Fish. Res. Board Can. 35: 754–765.

Une communauté intertidale riche et productive a été exposée continuellement durant 5 ans à de faibles quantités de fuel-oil « Navy Special » provenant du *General M.C. Meigs*, un transport de troupes désarmé qui s'est échoué à la côte de Washington en janvier 1972. Des observations faites sur les populations animales et végétales, et sur les modalités de leur absorption des hydrocarbures du pétrole démontrent qu'il y eut contamination dès le début de la période d'étude et que les effets du déversement persistèrent durant toute la période. Pendant au moins 1 an, nous avons observé des oursins de mer anormaux et morts, ainsi que la perte de frondes et de pigment par les algues dans des régions localisées du voisinage de l'épave. En moins de 2 mo après l'accident, des membres importants de la communauté avaient absorbé des hydrocarbures paraffiniques qui étaient encore présents chez certaines espèces même après 5 ans. Bien qu'on ait constaté des changements dans certaines espèces au début de cette faible pollution persistante, l'équilibre des communautés dans cet écosystème intertidal rocheux ne semble pas avoir été notablement altéré.

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THE unmanned troopship *General M.C. Meigs* (AP-116, 190 m length, 23 m beam, and 7.6 m draft), while under tow from Puget Sound to San Francisco, broke loose and came ashore on the northwest coast of the State of Washington on January 9, 1972. The hull was driven onto a rock pinnacle where it immediately broke into bow and stern sections forming a breakwater that protected "Wreck Cove" from the incoming waves. The fantail then separated from the stern section (Fig. 1). Subsequently the stern section has been progressively ripped apart by wave action until only the mast remains (Fig. 2). The bow section has withstood the pounding of the seas with only a slow loss of its superstructure.

Oil (Navy Special fuel oil) from ruptured fuel tanks

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FIG. 1. *General M.C. Meigs* aground at Portage Head off Wreck Cove 5 mo after the accident. Size is indicated by the two persons in a raft at lower center.

was released as the stern section of the hulk was ground down onto a submerged rock ledge running parallel to the beach. A major single oil spill occurred during the first 2 d following the accident, and most of the fluid oil came ashore on the shallow rock shelf and intertidal margins of Wreck Cove. Oil released in the form of discrete globules, up to 30 cm diam, has continuously floated into Wreck Cove where it becomes incorporated into the coarse sand beach. Fresh tar globules have been observed for over 5 yr although the individual size and abundance of the globules have diminished with time.

FIG. 2. Remains of the *General M.C. Meigs* 5 yr after the incident showing the relatively intact bow section (*left*) and the aft mast, which had apparently become wedged into a submerged rock reef (*right*). The grounding occurred on the Makah Indian Reservation; the headland in the upper left is being included in the Cape of Arches portion of the Olympic National Park, Washington.

The immediate and short-term effects of the oil contamination resulting from the *General M.C. Meigs* incident on the biota of this rocky shelf ecosystem have been reported by Clark et al. (1973). The recovery of the disturbed areas, as studied from January 1973 to August 1974, was reported in a second paper (Clark et al. 1975) and summarized in a review (Clark and Finley 1977). We are now reporting the results from over 5 yr of visual and chemical investigations of the impact of this persistent, low-level oil pollution on the cold-water marine environment at Wreck Cove. These investigations include assessment of the effects on animal and plant communities, the petroleum hydrocarbon uptake by certain species, and the weathering of the oil that had been released into the study area.

## Materials and Methods

### Animal Populations

Long-term changes in the occurrence and abundance of populations of animals and changes in their condition were studied for over 5 yr in Wreck Cove and nearby control areas by making 10 surveys during periods of the lowest monthly tide (Table 1). Methods of making the surveys have been reported (Clark et al. 1973, 1975) but are briefly reviewed here.

The procedure of a survey included (1) making a general inspection of the condition of animals throughout Wreck Cove; (2) enumerating prominent species at predetermined marked sites; (3) making a checklist of fauna found adjacent to a marked site; and (4) photographing prominent communities of animal life to record existing diversity and biomass. Surveys for evidence of oil contamination were confined to Wreck Cove because this was the primary area

TABLE 1. Field investigations at the site of the wreck of the *General M.C. Meigs*.

| | Jan. 11, 1972 | Feb. 9–10, 1972 | Mar. 15–16, 1972 | Apr. 18, 1972 | May 15–16, 1972 | June 12–13, 1972 | Aug. 9, 1972 | Oct. 20, 1972 | Jan. 17, 1973 | Feb. 12, 1973 | Mar. 9, 1973 | June 19, 1973 | July 27, 1973 | Oct. 25, 1973 | Feb. 4, 1974 | Aug. 19–20, 1974 | Feb. 16, 1977 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| General observations | | | | | | | | | | | | | | | | | |
| Visual overview/photographs | × | × | × | × | × | × | × | × | × | × | × | × | × | × | × | × | × |
| Biological survey | — | × | × | × | × | × | — | × | × | — | — | — | × | — | — | × | × |
| Animal population counts | — | — | × | × | × | × | — | × | × | — | — | — | × | — | — | × | × |
| Plant population observations | — | × | × | × | × | × | × | × | — | × | × | × | × | × | × | × | × |
| Hydrocarbon uptake sampling | — | × | × | × | × | × | × | × | × | × | × | × | × | × | × | × | × |

of impact. Observations of the area were made as identical as possible within limitations imposed by the duration of the low tide, the tide height, the height of the storm surf, and daylight. Priority was given to enumerating species at a marked site and making a checklist of fauna adjacent to these sites.

Fifteen sites were selected to include prominent species of animals in each of the intertidal zones of the shelf (Fig. 3). Sites were located along a profile from the upper intertidal (+1.7 m) seaward to the lower tidal zones of the shelf (−0.2 m) (Clark et al. 1973). Each site was marked with a nail driven into the rock substrate with a cement nailing gun. The nails were easily located over the 5-yr period using a map of the beach. Only two nail sites were lost during this period, probably from log or wood debris being dashed against the rock substrate by waves.

At each site, selected animals within a 30 × 30-cm ruled frame that was oriented with a compass were enumerated and photographed. Thus, identical areas at each site were studied on nine surveys. At sites where snails, limpets, and barnacles were studied, they were counted within a specified 10-cm$^2$ area within the frame, and mussels were counted within a specified 10 × 30-cm area.

The counting procedure entailed counting the numbers of live and dead small barnacles (*Chthamalus dalli* and *Balanus* sp.), large barnacles (*Balanus cariosus*), and mussels (*Mytilus californianus*) in the frames. Three to five replicate counts of mussels and barnacles were made at four sites to determine counting error. Abundance of the colonial anemone (*Anthopleura elegantissima*) within the frame at site no. 4 was determined by estimating the percent of the substrate they covered.

A checklist of species within 5 m of a site was compiled by identifying animals during the survey or by collecting samples and identifying them later in the laboratory. These species included common, uncommon, and cryptic types (Clark et al. 1973, 1975).

A rock substrate beach at Waatch Point (6.3 km north of Wreck Cove), which supports many of the animals that are present at Wreck Cove, was used as a control site (Clark et al. 1973). Observations (April 17 and June 12, 1972) made at this control site covered only gross aspects of the animal and plant community compared with the studies at Wreck Cove.

### PLANT POPULATIONS

A reference algae collection was made from a systematic search at most of the animal population study sites (Fig. 3: Sites 1, 2, 3, 4–7, 8–12, and 13–15). Thirty-seven prominent species of algae were identified (Clark et al. 1973). Initial damage to the plant community was divided into two categories: (1) physical changes to plant structure (loss of fronds) and oil coatings; and (2) chemical changes as evidenced by loss of color ("bleaching").

### PETROLEUM HYDROCARBONS

Dominant species of plants and animals that had been exposed to oil contamination from the *General M.C. Meigs* were analyzed and compared with unexposed control specimens of the same species. All samples were chilled for transportation to Seattle and frozen within 16 h. Methods of collection, preservation, preparation, and analysis of intertidal biological specimens to determine uptake of pollutant hydrocarbons have been previously described (Clark et al. 1973; Clark and Finley 1973a; Clark and Finley 1974).

Algae (*Bossiella* sp.) and goose barnacles (*Mitella polymerus*) were extracted after removal of epiphytic algae, endemic organisms, and excess surface water. Mussels were shucked while partly thawed; mussels and goose barnacles were homogenized between extractions (Clark and Finley 1973a). A sample consisted of 3–11 individual organisms depending on the species; weight of a sample varied from 40 to 60 g, wet weight.

The amounts of the *n*-paraffin ($n$-$C_xH_{2x+2}$) hydrocarbons were quantified by gas chromatography. Concentrations were expressed on a parts-per-billion (ppb: $10^{-9}$ g/g) dry extracted-weight basis: i.e. the weight of each individual *n*-paraffin divided by the sum of the weight of the dry residue remaining after removal of the organic material and the weight of the solvent extractables (Clark and Finley 1973a).

Tar globules (usually 4–6 cm diam and several centimetres thick) and scrapings from rocks were stored, chilled, wrapped in aluminum foil, and frozen on return to Seattle. The samples were extracted 3 times with pentane (approx. 1 mL each for sample sizes of 0.5–1.5 g of tarry residue), each time with 1–2-min exposure in an ultrasonic bath. The pentane-solubles were then chromatographed over silica gel and alumina as were the biological samples (Clark and Finley 1973a). The pentane-insoluble residue was then rinsed with three, 1-mL aliquots of 20% (vol/vol) methylene chloride in pentane; this material was chromatographed in a total of one bed volume of this solvent on the same

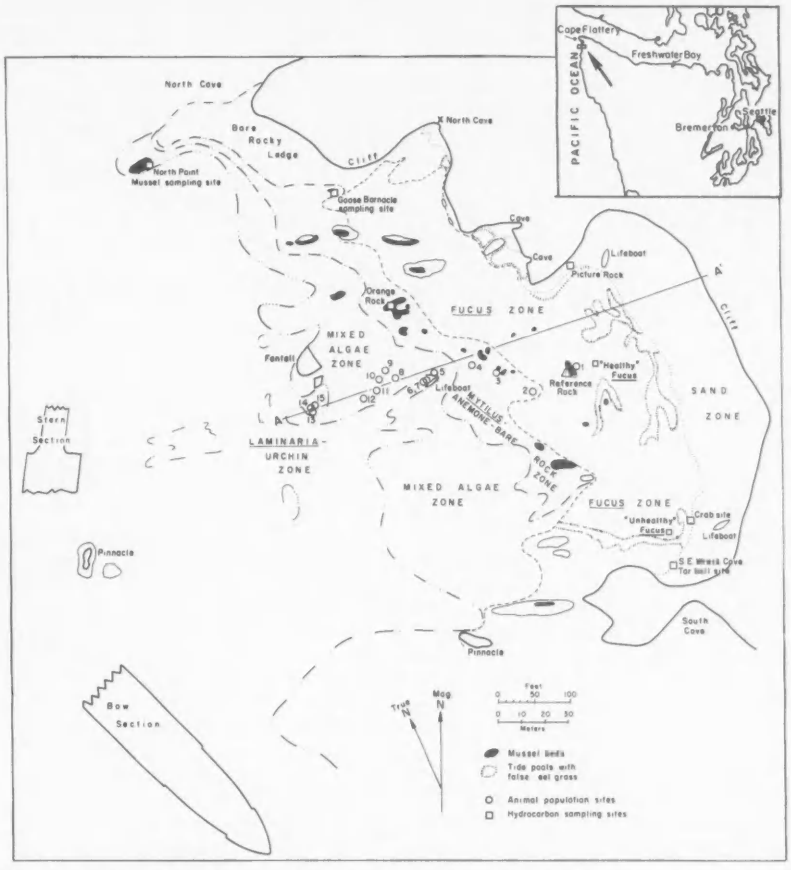


FIG. 3. Wreck Cove showing the animal population study sites and the hydrocarbon sampling sites.

column as for the pentane-solubles. A third set of rinsings followed using pure methylene chloride; the column was stripped with a fourth fraction by percolating through one bed volume of methanol. The last three fractions contained the unsaturated and aromatic hydrocarbons and the polar substances.

## Results

### ANIMAL POPULATIONS

The initial general survey in February 1972 provided no evidence of recent major damage or extensive animal mortalities. The expected indications of damage such as dead or dying animals, numerous empty mussel or barnacle shells, and bare rock areas showing vestiges of recent life were not evident. Analysis of the count data did not indicate differing ratios of live to dead barnacles or mussels during the period February 1972 to August 1974 (Clark et al. 1973, 1975) or as late as February 1977. However, the abundance of live barnacles, mussels, and the colonial anemones steadily declined from March 1972 to January 1973; the abundance of barnacles had increased by February 1977, whereas the other species remained at an unchanging low level (Fig. 4). Four other species did not have detectable or consistent changes in abundance (Fig. 4).

General surveys made throughout Wreck Cove did not disclose obvious damage to animals except to the purple sea urchin (*Strongylocentrotus purpuratus*). Damaged urchins were found inshore from the stern section at the 0.0 tidal level and below. In March 1972 these urchins had lost spines from the aboral surface, and by April some urchins were nearly aspinous. At some locations up to 70% of the urchins had lost spines from their aboral surface, and the occurrence of broken tests with tissue remaining indicated recent mortalities. Dead or abnormal urchins were not observed at the control sites. Damaged urchins were noted through July

1973, but they appeared normal on subsequent surveys.

None of the 45 animals observed in the species checklist (Clark et al. 1973, 1975) is known to have been eliminated from Wreck Cove or from a specific study site, nor were the numbers of species during any survey considered to be unexpectedly low. Analysis of the photographs did not reveal changes in animal populations.

## Plant Populations

Physical changes were severe for *Laminaria andersonii* in the area immediately inshore of the stern section; this alga had completely lost its fronds while the stipe and holdfast remained. This damage, which was initially observed several months after the grounding, was still apparent 2 yr later (Clark et al. 1975). Bleached algal tissue was observed within 6 mo of the grounding for a few species of Rhodophyta, notably the intertidal rock and tide pool dwelling plants *Corallina vancouveriensis*, *Prionitis lanceolata*, and *Ceramium* sp., as well as the spermatophyte *Phyllospadix scouleri* (false eel grass) (Clark et al. 1973).

During the summer of 1973 (1½ yr after the incident), the brown alga (*Hedophyllum sessile*) outgrew other algal species to become the dominant alga in the middle intertidal zone of Wreck Cove. By the summer of 1974 (2½ yr later), however, other algae such as *Halosaccion*, *Egregia*, *Desmarestia*, *Gigartina*, *Rhodoglossum*, *Iridaea*, *Codium*, and *Ulva* began to appear in an expected distribution pattern. During the last field observation (February 1977), the early spring development of all plant growth was progressing exceptionally well. False eel grass blades were green and healthy with no traces of necrosis. All new algal growth was crisp and regular. No degradation or attrition of fronds was observed nor was pigment loss noticeable in either thalli or coralline encrustations of the red algae.

## Hydrocarbon Concentrations in Organisms

Hydrocarbon patterns have been compared and interpreted for exposed and control samples of algae (*Fucus gardneri*, *Calliarthron schmittii*, and *Bossiella* sp.), false eel grass, purple sea urchins, goose barnacles, purple shore crabs (*Hemigrapsus nudus*), and mussels (Clark et al. 1973; Clark et al. 1975; Clark and Finley 1973b).

Moderate-level hydrocarbon uptake (Fig. 5) was reported for mussels collected and analyzed 2 mo after Navy Special fuel oil leaked from the *General M.C. Meigs* (Clark and Finley 1973b). The *n*-paraffin pattern of mussels collected at the Freshwater Bay control location (Fig. 3) is an average value of two composite samples collected in February of 1972 and 1973. This is assumed to represent the biogenic or environmental hydrocarbon baseline of the mussels. The *n*-paraffin pattern of the exposed organisms (Orange Rock, March 1972; Fig. 3) represents the biogenic baseline plus the

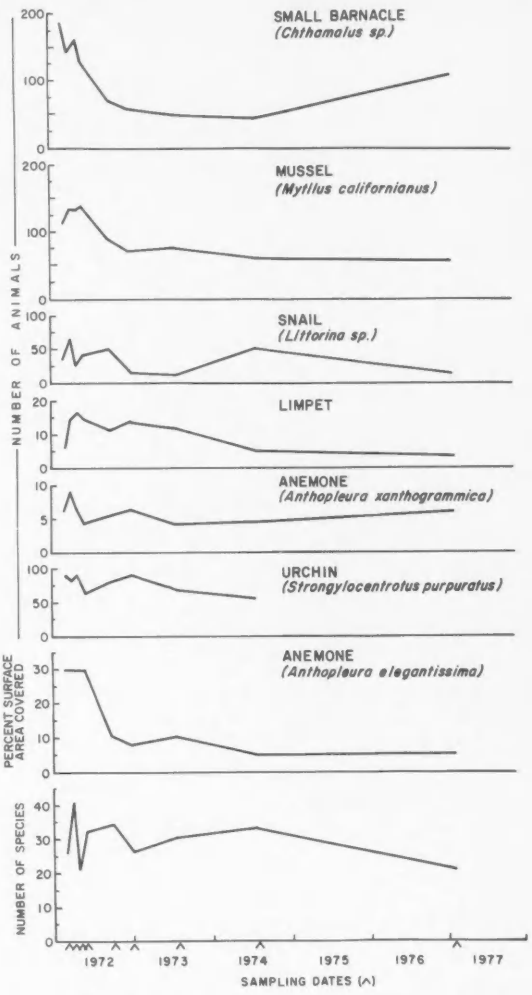


FIG. 4. Abundance of animals in numbers of individuals, percentage of surface area covered, and numbers of species at or near a sampling site during the 5-yr study. The numbers of small barnacles, mussels, snails, limpets, and anemones (*A. xanthogrammica*) are averaged for three to six sites.

pollutant uptake. If the *n*-paraffin pattern of the control samples is subtracted from that of the exposed organisms, the difference constitutes the residual, presumably petroleum-related, *n*-paraffin hydrocarbon pattern. The residual hydrocarbon pattern (Fig. 5B) calculated in this fashion for the mussels and the *n*-paraffin hydrocarbon pattern of a representative beach oil globule sample show similarities beyond *n*-$C_{18}$ in the configuration of their patterns (peaks at 20–21, 25, 28, 31, and 34–35 and the troughs at 19, 24, 26–27, 29, and 33). In addition, the residual hydrocarbon content of the

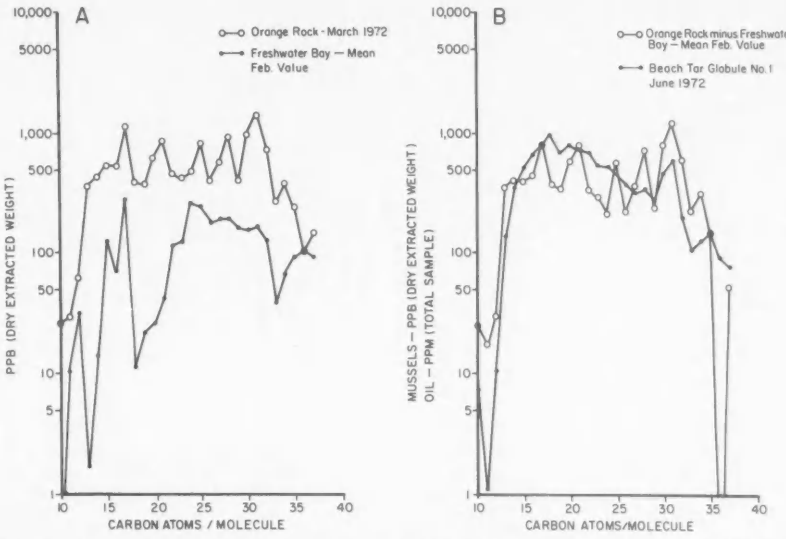
A
Orange Rock - March 1972
Freshwater Bay — Mean Feb. Value
PPB (DRY EXTRACTED WEIGHT)
CARBON ATOMS / MOLECULE
B
Orange Rock minus Freshwater Bay — Mean Feb. Value
Beach Tar Globule No. 1 June 1972
MUSSELS — PPB (DRY EXTRACTED WEIGHT)
OIL — PPM (TOTAL SAMPLE)
CARBON ATOMS/MOLECULE

FIG. 5. Paraffin hydrocarbon patterns for mussels (*Mytilus californianus*) exposed to moderate-level oil pollution. (A) Exposed mussels collected at Orange Rock 2 mo after the grounding of the *General M.C. Meigs* are compared with control mussels collected at Freshwater Bay; (B) the residual hydrocarbon pattern is compared with the Navy Special fuel oil residue pattern in a beach tar globule (No. 1, June 1972; Clark et al. 1975).

incorporated *n*-paraffins was from 100 to 1000 ppb. The total residual paraffin hydrocarbon ($n$-$C_{14-37}$) content in mussels was 11 ppm which is over 3 times the biogenic content of the controls.

Paraffin hydrocarbon patterns from mussels collected at Wreck Cove 5 yr after the accident are shown in Fig. 6. The Orange Rock samples had a total paraffin content (17 ppm, $n$-$C_{14-37}$) twice that of the nearby North Point mussel population (8 ppm); mussels from both areas had paraffin contents greater than the winter average of mussels from Freshwater Bay (3 ppm). The residual hydrocarbon content (Fig. 6B) of Orange Rock mussels was 14 ppm and of North Point mussels was 5 ppm. These residual patterns were similar to one of the two tar globules collected inshore of these sites during the same sampling period (peaks at 17, 21–22, 25, 30–31, and 35 and troughs at 19–20, 23, 26, and 32) although the similarity between the residual patterns and the pollutant is not as apparent as in the 2-mo sample (Fig. 5B).

Analyses of goose barnacles sampled in June 1972 (5 mo after the grounding) and in February 1977 (more than 5 yr after the grounding) at the same site (Fig. 3) showed different hydrocarbon patterns. The June 1972 sample displayed an *n*-paraffin hydrocarbon pattern and content (Fig. 7A) similar to those reported earlier (fig. 11 in Clark et al. 1973). The plot of the 1977 sample, however, shows a considerably different pattern — one that is more similar in shape (Fig. 7B: trough near 19–20 and broad peak at 25–29) and content (Table 2) to samples collected at a site (Mukkaw Bay) remote from known sources of pollution.

One coralline algal species (*Bossiella*, 48 g wet whole weight) collected 5 yr after the grounding had an *n*-paraffin pattern nearly identical to a control sample from Freshwater Bay (81 g wet whole weight; Clark et al. 1975). The analysis of only one other coralline alga, *Calliarthron schmittii* (65 g wet whole weight), obtained 1 mo after the grounding (Clark et al. 1975) suggested that within the 1st mo, the calcified alga did not appear to incorporate measurable concentrations of the pollutant, although other coralline species (such as *Corallina vancouveriensis*) had become bleached.

The unsaturated hydrocarbon fraction from the alumina–silica gel column chromatography separation step (fig. 10 in Clark and Brown 1977) eluted with 20% (vol/vol) methylene chloride in pentane was studied using capillary gas chromatography (MacLeod et al. 1976). Preliminary findings show that the *Bossiella* algae collected in February 1977 had a simple gas chromatogram of the unsaturated hydrocarbon fraction. This chromatogram contained only 46 well-resolved peaks over the retention time range from the methylnaphthalenes to beyond perylene (MacLeod et al. 1976) and displayed a baseline without any unresolved complex mixture envelope (Clark and Brown 1977). Fifteen of the peaks had no corresponding peaks on a complex-appearing gas chromatogram (133 peaks and an unresolved complex mixture envelope above the instrument baseline) seen in a tar globule (No. 1, February 1977) collected at the same time.

In contrast to the gas chromatogram of the rela-

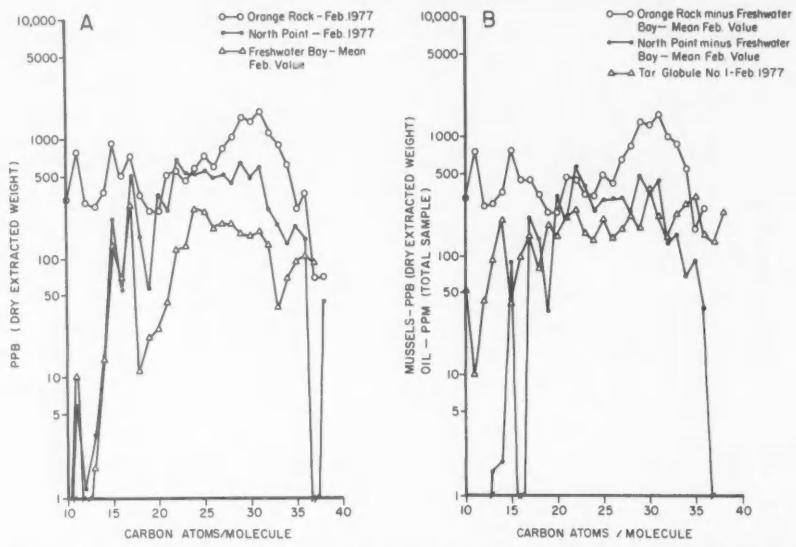
A
Orange Rock - Feb 1977
North Point - Feb 1977
Freshwater Bay - Mean Feb. Value
PPB (DRY EXTRACTED WEIGHT)
CARBON ATOMS/MOLECULE
B
Orange Rock minus Freshwater Bay - Mean Feb. Value
North Point minus Freshwater Bay - Mean Feb. Value
Tar Globule No 1-Feb 1977
MUSSELS - PPB (DRY EXTRACTED WEIGHT) OIL - PPM (TOTAL SAMPLE)
CARBON ATOMS / MOLECULE

FIG. 6. Paraffin hydrocarbon patterns for mussels (*M. californianus*) collected 61 mo (Feb. 1977) after the grounding of the *General M.C. Meigs*. (A) Samples collected at Orange Rock and North Point are compared with mean February values of mussels from the Freshwater Bay control site; (B) the residual hydrocarbon patterns are compared with a fresh tar globule (No. 1) collected in Feb. 1977.

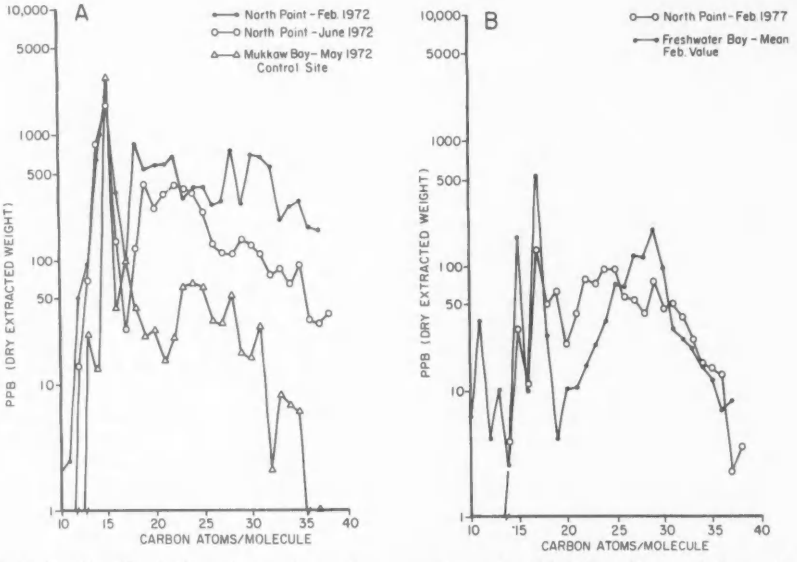


FIG. 7. Paraffin hydrocarbon patterns for goose barnacles (*Mitella polymerus*) from the *General M.C. Meigs* sites are compared with patterns from control samples. (A) Exposed samples from North Point (Feb. and June 1972) are compared with a control sample from Mukkaw Bay, 3 km north (May 1972; Clark et al. 1973); (B) sample from North Point (Feb. 1977) is compared with mean February values of goose barnacles from the Freshwater Bay control site.

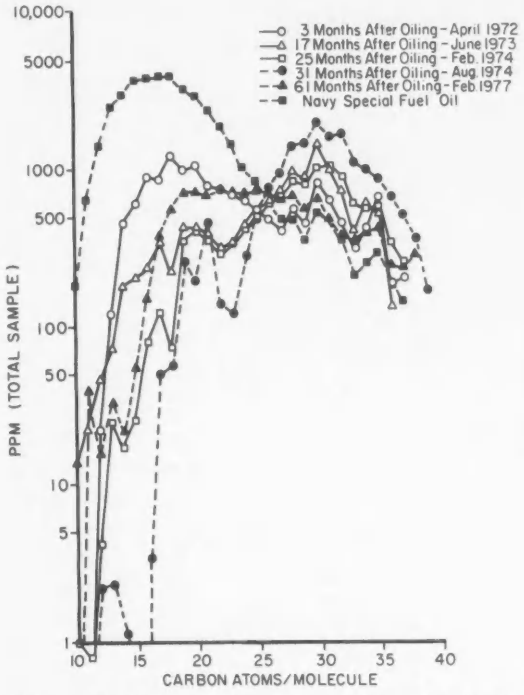


FIG. 8. Paraffin hydrocarbon patterns of Navy Special fuel oil residues after several periods of weathering at Picture Rock.

tively uncomplicated unsaturated hydrocarbon fraction of the alga, the gas chromatograms of the mussel samples collected over 5 yr after the accident displayed complex patterns containing many peaks, unresolved complex mixture envelopes above baseline, and other similarities to tar globule chromatograms. For instance, the Orange Rock mussels had 118 peaks in the methylnaphthalenes–perylene region and the North Point mussels had 107 peaks compared to 133 peaks in the tar globule (No. 1, February 1977). Sixty-seven of the peaks in the Orange Rock mussel chromatogram and 78 in the North Point sample corresponded to peaks in the tar globule.

Extracts from goose barnacles collected 5 mo after the incident gave a gas chromatogram with 134 major peaks appearing above an unresolved complex mixture envelope. A tar globule (No. 2, June 1972) contained 137 major peaks, 123 of which also appeared in the goose barnacle chromatogram. A solvent blank had only three major peaks in the methylnaphthalenes–perylene region. Extracts from goose barnacles collected 61 mo after the initial spill showed no obvious similarity to the tar globules sampled at that time. This recent (February 1977) goose barnacle chromatogram showed a simple pattern with no unresolved complex mixture envelope and a total of only 26 peaks in the methylnaphthalenes–perylene region. All of these peaks corresponded in retention times to peaks in the earlier goose barnacle sample (June 1972) but correlated with only five of the peaks in a tar globule (No. 1, February 1977), which suggests that the recent goose barnacle sample had little, if any, petroleum-related unsaturated hydrocarbon content.

These preliminary findings based on gas-chromatographic retention times are being supplemented by gas-chromatographic–mass spectrometric (GC–MS) examinations of the major individual components using a peak-by-peak correlation between organism chromatograms and tar globule chromatograms. This effort is currently in progress and results will be reported in the future. The results thus far from the unsaturated hydrocarbon fractions, however, closely complement and support the *n*-paraffin hydrocarbon data: (1) the *Bossiella* alga (February 1977) did not display measurable petroleum hydrocarbon uptake; (2) the mussels collected over 5 yr after the accident (Orange Rock and North Point) displayed *n*-paraffin hydrocarbon uptake and complex gas chromatograms similar to tar globules; (3) the goose barnacles collected 5 mo (June 1972) after the accident had *n*-paraffin patterns very similar to previously reported contaminated organisms and the unsaturated hydrocarbon chromatogram had a complex pattern similar to a tar globule collected at that time; and (4) the more recently collected goose barnacles (February 1977) had an *n*-paraffin pattern similar to uncontaminated control samples and a relatively simple (nonpetroleum) unsaturated hydrocarbon gas chromatogram.

## Picture Rock Tarry Residues

In the 2 d following the grounding, near-hurricane conditions caused considerable amounts of released oil to be deposited on a rock (Picture Rock, Fig. 3) located about 2.8 m above mean lower low water in the northeast extreme corner of Wreck Cove. Since the oil was well above the usual tide line and only occasionally exposed to the splash from winter storms, oil residues have remained in the cracks and crevices (1–10 mm depth) of the rock for the entire period of observation.

Chemical analyses of the *n*-paraffin hydrocarbon components (Fig. 8) have shown the usual loss of low molecular weight hydrocarbons (Blumer and Sass 1972), but the loss we observed was slow (Clark et al. 1975). The total *n*-paraffin content of the tar scrapings (Table 2) varied between samples as some samples from Picture Rock were thicker than others, depending on crevice depth, and may have weathered less rapidly than thinner scrapings. The pattern for residues collected over 5 yr after the initial oiling showed a distinct loss of lower molecular weight paraffin hydrocarbons and a general smoothing of the paraffin curve over the $n$-$C_{20}$ to $n$-$C_{35}$ region.

TABLE 2. Normal paraffin hydrocarbon data analysis of animals and fuel oil residues from the *General M.C. Meigs* oil spill[a].

| Sample analyzed Location collected | Sampling period | Solvent extractables (ppm × $10^3$) | Total *n*-paraffin hydrocarbons $C_{14-37}$ ppm | *n*-$C_{17}$: pristane ratio | Major hydrocarbon | | *n*-$C_{18}$ ratio | $CPI_{14-20}$[b] | $CPI_{20-36}$[b] | Unresolved envelope: *n*-$C_{17}$/background |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Mussels (Mytilus californianus)* | | | | | | | |
| Orange Rock (exposed) | Mar. 1972 | 160 | 14 | 1.4 | $C_{31}$ | 10% | 26 | 1.4 | 1.1 | 1.3 |
| Orange Rock (exposed) | Feb. 1977 | 120 | 17 | 2.1 | $C_{31}$ | 10% | 33 | 1.6 | 1.1 | 3.2 |
| North Point (exposed) | Feb. 1977 | 140 | 7.9 | 1.2 | $C_{22}$ | 9% | 150 | 2.4 | 1.1 | 3.1 |
| Freshwater Bay (control) | Feb. Mean | 120 | 2.9 | 2.4 | $C_{17}$ | 10% | 42 | 4.4 | 0.9 | 1.7 |
| | | | *Goose Barnacles (Mitella polymerus)* | | | | | | | |
| North Point (exposed) | Feb. 1972 | 24 | 30 | 0.09 | $C_{15}$ | 15% | 21 | 1.2 | 1.3 | 4.9 |
| North Point (exposed) | June 1972 | 23 | 6.5 | 0.02 | $C_{15}$ | 14% | 45 | 3.0 | 1.1 | 18 |
| North Point (exposed) | Feb. 1977 | 17 | 1.1 | 0.05 | $C_{24}$ | 8% | 97 | 3.0 | 1.1 | 37 |
| Mukkaw Bay (control) | May 1972 | 30 | 3.6 | 0.01 | $C_{15}$ | 83% | 85 | 1.1 | 28 | 130 |
| Freshwater Bay (control) | Feb. Mean | 15 | 1.7 | 0.23 | $C_{17}$ | 32% | 170 | 16 | 1.3 | 76 |
| | | | *Petroleum residues*[c] | | | | | | | |
| Tar globule | Feb. 1972 | 95 | 7 500 | 1.2 | $C_{14}$ | 6% | 16 | 1.0 | 1.1 | 2.6 |
| Tar globule No. 1 | June 1972 | 670 | 8 500 | 0.96 | $C_{18}$ | 9% | 18 | 0.8 | 0.9 | 2.0 |
| Tar globule No. 2 | June 1972 | 160 | 9 400 | 1.2 | $C_{20}$ | 8% | 17 | 1.1 | 1.1 | 2.3 |
| Tar globule | Feb. 1973 | 200 | 11 000 | 0.94 | $C_{20}$ | 7% | 20 | 1.1 | 1.0 | 2.1 |
| Tar globule | Oct. 1973 | 130 | 7 600 | 0.81 | $C_{20}$ | 7% | 19 | 1.1 | 1.1 | 1.9 |
| Tar globule No. 1 | Feb. 1977 | 140 | 4 900 | 0.50 | $C_{30}$ | 8% | 50 | 1.1 | 1.0 | 1.6 |
| Tar globule No. 2 | Feb. 1977 | 1100 | 150 000 | 2.5 | $C_{29}$ | 8% | 28 | 1.1 | 1.1 | 9.6 |
| Picture Rock | Apr. 1972 | 600 | 15 000 | 2.3 | $C_{18}$ | 8% | 17 | 0.9 | 1.0 | 2.5 |
| Picture Rock | June 1973 | 530 | 12 000 | 1.6 | $C_{30}$ | 12% | 50 | 1.2 | 0.9 | 2.4 |
| Picture Rock | Feb. 1974 | 490 | 11 000 | 0.3 | $C_{31}$ | 9% | 140 | 1.9 | 1.0 | 2.2 |
| Picture Rock | Aug. 1974 | 500 | 17 000 | 0.1 | $C_{30}$ | 12% | 4700 | 3.0 | 0.9 | 1.8 |
| Picture Rock | Feb. 1977 | 147 | 12 000 | 1.2 | $C_{22}$ | 6% | 80 | 1.2 | 1.0 | 2.4 |
| Navy Special fuel oil | 1971 | 950 | 40 000 | 1.6 | $C_{18}$ | 10% | 10 | 1.0 | 1.0 | 3.2 |

[a]All data rounded to two significant figures. A detailed discussion of the application of the parameters presented in this table has been presented by Clark and Finley (1973a).
[b]Carbon preference index (Clark and Finley 1973a).
[c]Pentane-soluble material.

## OIL GLOBULES IN THE INTERTIDAL ZONE

Tar globules that appeared fresh were collected in Wreck Cove 5 yr after the accident. However, the Washington coast has been subjected to numerous occurrences of small-scale tar ball pollution in the late spring for the past several years. We were, therefore, interested in whether or not the few tar globules we observed inshore from the *General M.C. Meigs* were still being released from the hulk or if they originated from other sources. Two samples collected in February 1977 had a very similar outward appearance; however, the *n*-paraffin hydrocarbon patterns and content were very different (Fig. 9). Tar globule No. 1 had a slightly saw-toothed hydrocarbon pattern; a comparable amount of solvent extractables; *n*-paraffin concentration range of 100–800 ppm; pattern peaks at 25, 30, 35, and 38; pattern troughs at 24, 32, and 36; and carbon preference indices (Table 2) similar to other beach tar samples (fig. 6 in Clark et al. 1975). Tar globule No. 2 collected in Southeast Wreck Cove in February 1977 had 30 times the *n*-paraffin pattern hydrocarbon content and a much smoother *n*-paraffin pattern than tar globule No. 1 (very little of the sawtooth shape), with a broad gradually increasing plateau between *n*-$C_{16}$ and *n*-$C_{29}$ followed by a rapid logarithmic decrease beyond *n*-$C_{29}$.

During every survey of Wreck Cove over the 5-yr period, we observed tar globules that frequently contained pieces of rock, shell, wood, evergreen tree needles, and miscellaneous fibrous materials, both internally and on the surface. Of the 15 tar globules analyzed, *n*-paraffin patterns were similar with the exception of one sample collected in February 1974 (Clark et al. 1975) that did not display the usual smooth curve below

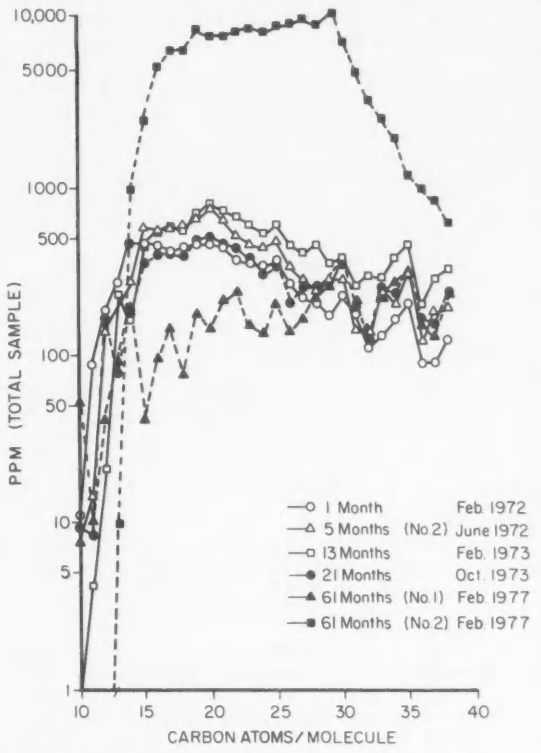


FIG. 9. Paraffin hydrocarbon patterns of fresh oil globules collected in southeast Wreck Cove over a 5-yr period.

$n$-$C_{22}$ and the one collected in February 1977 described above. The other tar globule (No. 1) collected in February 1977 was somewhat intermediate in character showing some loss of $n$-paraffin hydrocarbons below $n$-$C_{25}$ and a slight saw-toothed pattern below $n$-$C_{20}$.

## Discussion

### Biological Populations

The grounding of the troopship *General M.C. Meigs* and the resulting oil pollution exposed sessile animals and plants in Wreck Cove on the Washington coast to oil slicks and tar globules from January to June 1972. After this period, tar globules appeared to be the principal form of contamination throughout the study.

Changes in the number, occurrence, or overt condition of animals in Wreck Cove were not obvious for the 5 yr following the oil spill incident except in the case of the urchins, small barnacles, mussels, and the colonial anemone. Variations that did occur could have been the result of natural fluctuations (Dayton 1971), or changes in the ecology of Wreck Cove caused by the breakwater effect of the remaining hulk. If the oil pollutant had any effect on the animal populations, it was too subtle or localized to be detected by our methods except in the case of the purple sea urchin.

Observations on the damage to the sea urchin coupled with studies on hydrocarbon uptake suggest that oil pollution had affected this animal. During the first 10 mo of observations following the accident, many urchins in localized areas were dead or had lost enough of their spines to make their survival improbable. The behavior of urchins "decorating" their backs with available material of small size in the tide pools resulted in extensive use of fragments of false eel grass for such decoration. False eel grass was one of the species of plants that became coated with oil immediately after the grounding; large quantities of eel grass fragments spotted with tarry residues or bleached white were awash in tide pools in Wreck Cove. Consequently, about 80% of the urchins used false eel grass for decorating themselves immediately after the accident. By August 1974 only about 5% of the urchins still used the false eel grass for decoration; at that time, the eel grass appeared green and healthy. Based on our observations and on the hydrocarbon uptake data (Clark et al. 1975) we can conclude that the only logical reason for damage to urchins was from the oil contamination caused by the *General M.C. Meigs*.

The oil contamination-related damage and deaths of the purple sea urchin were not widespread, and they were outside the site transect (Fig. 3). Therefore, one must not discount the possibility that other less prominent species could have been affected but not detected by our study methods. It is also possible that the behavior of the urchin in decorating itself with pieces of contaminated plant material make it a unique and special case predisposing it to take up considerably more oil pollutants than other animals.

Several species of intertidal plants were seriously affected for several years in Wreck Cove as evidenced by physical changes (loss of fronds and coating with tarry residues) and by chemical changes (loss of pigment and uptake of petroleum paraffin hydrocarbons). Based on the observations from the *General M.C. Meigs* and on other oil pollution studies (Smith 1968; Nelson-Smith 1971; Baker 1971; Baker et al. 1976), intertidal algae may serve as one of the medium term (1–4 mo after the initial short-term animal mortality phase) indicators of petroleum pollution.

### Chemical Uptake

Our analyses demonstrated that petroleum hydrocarbon residues were present in certain marine animals for at least 9 mo after the initial wreck and oil spill, whereas in specific algal species residues remained for more than a year (Clark et al. 1975). We have now found mussels collected over 5 yr after the accident that contain similar hydrocarbon residues.

The long-term occurrence of contamination in organisms is possibly the result of their periodic recon-

tamination by the tar globules in combination with the persistence of the original large petroleum uptake immediately after the incident. Different species have different mechanisms for accumulation and release of oil (Varanasi and Malins 1977), a fact that might explain the difference in the paraffin hydrocarbon patterns found in goose barnacles compared with mussels, both sampled 5 yr after the accident. We suspect that recontamination by winter discharges of tarry material from the hulk caused the high levels in the mussels. The hydrocarbon pollutant patterns represented by the residual paraffin plots of the organisms reflect the degree of pollution throughout the sampling period.

The weathering of oil residues is dependent on the variations of environmental conditions to which the oil is exposed (Clark and MacLeod 1977); however, most of the weathered oil we sampled showed a basic uniformity in *n*-paraffin hydrocarbon patterns to Navy Special fuel oil.

For instance, the tarry residue on Picture Rock was very slow to disappear because of its location in the upper splash zone, where it cannot be routinely attacked by microorganisms that require an aqueous medium for growth (Karrick 1977). Presumably, the tough coating would be hard to penetrate and would reduce evaporation. The usual loss of low molecular weight hydrocarbons was expected, like that reported by Blumer et al. (1973) for a weathered oil sample on a warm and dry rock on Martha's Vineyard, Mass., which was studied for 15 mo.

We believe that throughout our 5-yr survey we have found newly released residues of Navy Special fuel oil from the *General M.C. Meigs* and that the reasonable similarity of the original paraffin hydrocarbon patterns of the oil to subsequent patterns supports this belief. However, one tar globule collected in February 1977 (tar globule No. 2) appeared to be very different chemically from the other 14 tar globules analyzed over the 5-yr study period. Either a tank containing a very different oil had been ruptured during the winter of 1977 or this globule came from some source other than the *General M.C. Meigs*.

Although the *General M.C. Meigs* incident was a relatively minor spill, based on initial oil loss and type of petroleum involved (Clark and Finley 1977), tangible evidence of damage and pollutant uptake by the intertidal organisms was observed. This emphasizes the sensitivity and vulnerability to pollution of those organisms that are generally considered hardy by virtue of the environmental stresses they normally encounter.

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# Recovery of Benthic Macrofauna from Chronic Pollution in the Sea Area off a Refinery Plant, Southwest Finland[1]

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LEPPÄKOSKI, E. J., AND L. S. LINDSTRÖM. 1978. Recovery of benthic macrofauna from chronic pollution in the sea area off a refinery plant, southwest Finland. J. Fish. Res. Board Can. 35: 766–775.

Quantitative field studies (density, wet weight biomass, Shannon diversity, species richness, evenness of distribution) on benthic sublittoral macrofauna were made in the vicinity of an oil refinery in southwest Finland before and after the installation of a new wastewater treatment plant that reduced the amount of oil and liquid effluents by ca. 90–95%. The number of species and species diversity increased during the 1st and 2nd yr after pollution abatement at the stations close to the former outflows. The amphipods *Pontoporeia affinis*, *Corophium volutator*, and *C. lacustre*, midge larvae of the *Chironomus plumosus*-group, the oligochaete *Tubifex costatus*, the polychaetes *Harmothoe sarsi* and *Polydora redeki*, and the bivalve *Cardium* sp. were the most successful recolonizers of the 23 taxa sampled. The strong lethal effect of oil-contaminated sediments upon *Chironomus plumosus* larvae decreased markedly in laboratory experiments (LT50 was estimated at 7 d in 1973 and at 28 d in 1974; in 1975, 80–90% of the larvae survived for 28 d). Details of postabatement succession are discussed. The results demonstrate not only the recovery from chronic oil pollution but also the degree of ecological damage caused by previous continuous discharge of oil.

*Key words:* Baltic Sea, *Chironomus plumosus*, macrobenthos, oil pollution, pollution abatement, recolonization, sediment biotests

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Nous avons poursuivi des études quantitatives sur le terrain (densité, biomasse en poids humide, nombre et diversité des espèces, uniformité dans la répartition) sur la macrofaune sublittorale benthique au voisinage d'une raffinerie de pétrole dans le sud-ouest de la Finlande avant et après l'installation d'une nouvelle usine de traitement des eaux usées qui diminua d'environ 90 à 95% le volume des effluents de pétrole et autres liquides. Le nombre et la diversité des espèces augmentèrent pendant les deux années qui suivirent l'élimination de la pollution aux stations voisines des anciennes bouches de sortie. Sur les 23 taxons échantillonnés, les amphipodes *Pontoporeia affinis*, *Corophium volutator* et *C. lacustre*, les larves du groupe *Chironomus plumosus*, l'oligochète *Tubifex costatus*, les polychètes *Harmothoë sarsi* et *Polydora redeki*, et le bivalve *Cardium* sp. sont les nouveaux colonisateurs qui ont le mieux réussi. L'action hautement létale des sédiments contaminés par le pétrole sur les larves de *Chironomus plumosus* diminua notablement dans des expériences de laboratoire (on a estimé la TL50 à 7 jours en 1973 et 28 jours en 1974; en 1975, de 80 à 90% des larves survécurent pendant 28 jours). Nous examinons en détail la succession des espèces après élimination de la pollution. Les résultats démontrent non seulement le rétablissement après une pollution chronique par le pétrole mais aussi l'étendue des dommages écologiques causés par les effluents antérieurs de pétrole.

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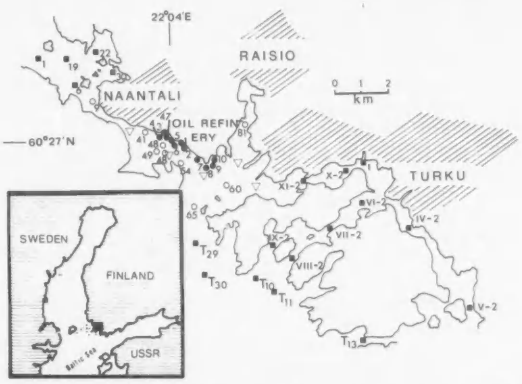


FIG. 1. Sampling sites. (●) = inshore station in the vicinity of the oil refinery, (○) = intermediate station, (■) = control station, and (▽) = hydrographical station. Station codes as used in various local surveys since 1965.

THE noxious effects of oil pollution on marine life have been investigated mostly in connection with accidental gross spillages. Chronic effects, caused by small but repeated spillages in harbors or by continuous low-level discharges of oil industry effluents, have been studied much less thoroughly (see Reish 1965; Crapp 1971; Baker 1973; Boesch et al. 1974; Wharfe 1975). In addition, refineries are often situated in the vicinity of other industrial plants or cities. Therefore, the effect and ecological impact of refinery effluents alone as a part of multisource pollution are not easily assessed. Most studies on accidental oil pollution of the sea (reviewed by GESAMP 1977) deal with the littoral biota. The fractions of oil discharged from a refinery are, however, already effectively dispersed when released in the cooling water stream. A certain amount of this oil reaches the bottom, absorbed to mineral, detritus, or other oleophilic particles and may affect the sublittoral biota.

In the present study it was possible to investigate the bottom fauna in an area where most of the oil pollution was eliminated, while the other sources of pollution remained unaltered. Simultaneous laboratory experiments were performed to simulate the effects of oil-loaded bottom substrates on some selected benthic invertebrates. The aim of this paper is to describe effects of chronic oil pollution on soft-bottom fauna in brackish water near an oil refinery at Naantali, southwest Finland (Fig. 1) in 1972–75, and the recovery after oil discharge abatement.

## Study Area

The refinery (operated by Neste OY; refining $2.9 \times 10^6$ tons/yr of crude oil) started in 1957. The oil harbor has been in operation since 1946. Until 1972 oily wastewaters were treated by mechanical separators only and discharged together with cooling water. The total oil discharge was estimated to be 150–300 kg/d. Since January 1973 all discharges have been led to a four-stage treatment plant (Fig. 2) in which (a) floating oil is mechanically separated, (b) chemical flocculation/flotation is induced by adding $FeSO_4$, followed by (c) biological treatment in an activated-sludge plant, and (d) an oxidation pond. The "once-through" cooling system was replaced in 1973 by a closed water circulation system with double heat exchangers. As a result total wastewater and oil discharges were reduced to less than one tenth of the earlier level. According to water control authorities, the average oil loading was about 30 kg/d in 1974 and 10 kg/d in 1975. The phenol content of discharged water has been on average $<1$ kg/d.

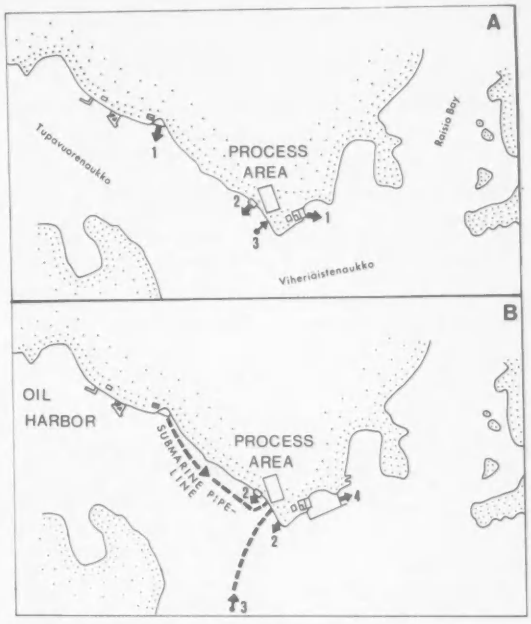


FIG. 2. Discharges from the oil refinery at Naantali in the 1960s (A) and since 1972–73 (B). Outflows from mechanical separators (1), outflow (2), and intake (3) of cooling water, and outflow from the new treatment plant (4).

The hydrography, pollution, and benthic fauna of the area have been investigated by several authors since 1960 (see Leppäkoski 1975a). Water quality has been fairly constant during the investigation period 1972–75 (Table 1). Mean salinity was 6.1‰ $S$ ($n = 76$; range 5.4–6.7‰) at depths of 10–15 m. Differences in the mean salinity values between successive years were 0.2–0.3‰. Annual temperature maxima (14–18°C) at this depth are usually recorded in September. The area is covered by ice for about 3½ mo. The lowest $O_2$ saturation value at 20 m deep during 1970–75 was 43% at localities in the vicinity of the refinery, regularly monitored by water control authorities. Minimum $O_2$ contents are usually recorded during late summer.

## Materials and Methods

The sampling stations (Fig. 1) can be divided into three groups: (1) 19 control sites, located 3 km or more from the refinery outfall; all these sites were sampled in 1970 (Lep-

TABLE 1. Water quality at Station 60 (Fig. 1) in August 1969–73 according to the measurements made by the local water control authorities (Turku Water District). Values are expressed as means ± SE.

| | Depth (m) | |
|---|---|---|
| | 1 | 13 |
| Transparency (cm) | 145 ± 20 | |
| Suspended matter (mg/L) | 8.3 ± 1.4 | 8.9 ± 1.9 |
| $O_2$ saturation (%) | 99 ± 9 | 37 ± 18 |
| Total phosphorus (μg/L) | 54 ± 3 | 450 ± 200 |
| Total nitrogen (μg/L) | 720 ± 140 | 830 ± 200 |
| Conductivity ($H_2O$) (μS/cm) | 9200 ± 310 | 9400 ± 550 |
| Chemical oxygen demand (mg/L) | 6.2 ± 0.9 | 5.3 ± 1.0 |
| Enterococci (exx/100 mL) | 33 ± 30 | |

päkoski 1972), only 10 in 1972, and all in 1975 (Juuti and Leppäkoski 1976); (2) 9 intermediate sites 400–1500 m from the outfall, sampled in 1970 and 1972–75; and (3) 11 inshore stations in the immediate vicinity of the refinery, sampled in 1972–75. Intersecting landmarks were used for position finding.

In most cases five replicate samples were taken at each station in August 1972–75 with a van Veen grab (0.05 $m^2$) and washed through a 0.8-mm mesh sieve. All material retained was preserved in 4% formalin, neutralized with hexamine, and stained with Rose Bengal. The animals were sorted from the sieving residue under a binocular microscope. All weights are given as formalin wet weight including mollusc shells. The material is deposited in the collections of the Archipelago Research Institute.

To characterize the faunal assemblages, three indices were calculated on the basis of species number and density values: Shannon's diversity index, $H'$ (Shannon and Weaver 1963); species richness, SR (Margalef 1958); and evenness, $J'$ (Pielou 1966).

For laboratory biotests with red midge larvae (*Chironomus plumosus*-group), the topmost 2 cm of sediment from several (up to 10) samples were taken with an Ekman-Lenz grab or with a modified 0.1-$m^2$ van Veen grab, the upper windows of which could be opened for subsampling the sediment surface. These sediment samples were sieved through a 0.8-mm mesh sieve and homogenized for use as experimental substrates. Control substrates as well as test animals were taken approximately 25 km from the refinery. Animals were held in 7 × 7 × 4 cm dishes filled with a 2-cm substrate layer. The dishes were kept in darkness in aerated brackish-water aquaria at +10°C.

Experimental oil-contaminated substrates were made by mixing known amounts (expressed as percent of wet sediment weight) of distillation bottom (boiling point above 350°C) from the refinery at Naantali, in natural sediment.

Test animals were acclimatized in uncontaminated sediment for 2 d or more before starting the experiments. They were observed daily during the 1st wk of each experiment and once a week later on. To examine the animals, the test substrate was gently passed through a sieve. After observation the animals were immediately returned to the same substrate. Larvae were considered dead when no movement was observed after manual irritation with a glass rod.

One-litre water samples taken at five stations (Fig. 1) by

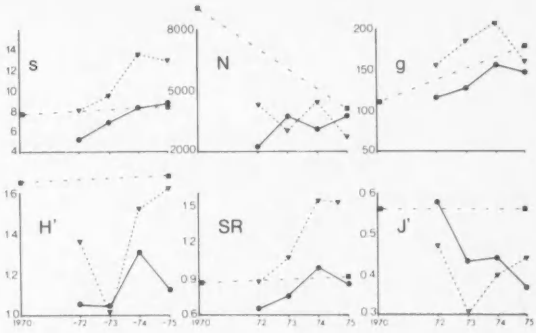


FIG. 3. The bottom fauna at the inshore stations (–▼–▼–; $n = 53$), intermediate stations (–●–●–; $n = 35$), and control stations (–■–■–; $n = 90$) in 1972–1975. $S$ = the number of species/locality, $N$ = the number of individuals/$m^2$, g = wet weight biomass g/$m^2$, $H'$ = Shannon diversity, SR = species richness index, $J'$ = evenness index. All values are arithmetic means.

water control authorities were treated with $CCl_4$ and filtered through an adsorption column of $Al_2O_3$. The absorbance of the extract at 342 and 350 nm was measured with an IR-spectrophotometer. Standard mixtures of isooctane, *n*-hexadecane, and benzene were used for calibration.

Substrate samples for analysis of total oil content were taken by a grab, homogenized immediately by vigorous stirring, and deep-frozen at −30°C. The samples were then suspended in water and boiled with $CCl_4$ for 2 h; the extract (shaken with $Al_2O_3$–Fluorisil) was analyzed by IR-spectrophotometry (calibrated according to API 733-58). The oil content is given as milligrams per kilogram sediment dry weight. All oil analyses were made by the environmental protection laboratory of Neste OY in Naantali.

## Results

### OIL IN WATER AND SEDIMENTS

At the five hydrographic stations where oil content of the surface water layer (0–1 m) has been determined since 1971, about 50% of the values were 0.1 mg/L or less but occasionally as high as 1.6–1.9 mg/L. Concentrations at stations east of the refinery were generally higher. It should be noted that not only the industrial plants, including the refinery, are responsible for the oil contamination observed; intensive sea traffic might also have an effect. The area is used for transport of refined oil as well as by car ferries and cargo vessels. No significant changes were noted in the oil content of the water ($\bar{x} = 0.20 \pm 0.03$ mg/L, $n = 62$ vs. $\bar{x} = 0.22 \pm 0.02$ mg/L, $n = 74$) after the new treatment plant started at the refinery.

Oil contents in the topmost substrate layer (about 0–10 cm) decreased at most of the sampling stations during the period 1972–75. Values exceeding 1000 mg/kg sediment dry weight were measured at Stations 2, 3, 7, 8, 9, and 10. Maximum values (>3000 mg/kg) were recorded at Station 7. At the intermediate sites (Stations 41–65) the sediment oil contents were <600

TABLE 2. The macrobenthic fauna at Station 48 off Naantali harbor in 1965–75 (in part according to Leppäkoski 1975a), and in 1976 (T. Juuti personal communication).

| | 1965 | 1967 | 1970 | 1972 | 1973 | 1974 | 1975 | 1976 |
|---|---|---|---|---|---|---|---|---|
| No. of species | 4 | 4 | 4 | 3 | 4 | 5 | 9 | 9 |
| Density (ind/m²) | 250 | 530 | 400 | 70 | 1650 | 1470 | 2634 | 1970 |
| Biomass (g/m²) | 6.5 | 7.9 | 17.9 | 0.4 | 27.6 | 29.6 | 157.4 | 282.5 |
| Diversity ($H'$) | 1.6 | 1.3 | 1.2 | 1.2 | 0.9 | 0.7 | 0.9 | |
| Species richness | 0.5 | 0.5 | 0.5 | 0.5 | 0.4 | 0.7 | 1.0 | |
| Evenness | 0.8 | 0.7 | 0.6 | 0.8 | 0.4 | 0.3 | 0.3 | |

mg/kg. The 12 inshore and intermediate localities (Stations 2–10, 47, 48, and 60) gave the following mean annual values (milligrams per kilogram): 1972, 1010; 1973, 920; 1974, 600; 1975, 530.

The intense bituminous odor that characterized about 90% of the grab samples taken in this area in 1965–67 (Leppäkoski 1975a) has totally disappeared during the 1970s.

## BOTTOM FAUNA

At the 19 control stations (>3 km from the refinery), the number of benthic species, species diversity, species richness, and evenness were similar in 1975 when compared with data from 1970 (Fig. 3). However, there was a marked decrease in the number of individuals during these years mainly due to the diminished abundance of tubificid worms (especially *Peloscolex heterochaetus*) and the clam *Macoma baltica*. The latter species contributed 90% of the increase of the total benthic biomass, expressed as wet weight in Fig. 3. In 1972, 10 of these control localities were sampled (Juuti and Leppäkoski 1976). No major changes in the faunal assemblages were observed except that the bottom fauna of the deep (25 m) sound in the intermediate area had been greatly impoverished (Table 2). Since 1972 the fauna at these depths, as well as that of the intermediate area in general, has improved (Fig. 3).

The macrobenthos in the inshore area near the former refinery outflows underwent a marked increase in species number, richness, and diversity during 1973–74, as shown in Fig. 3. A total of 23 taxa were sampled at these 11 stations (Fig. 4). Of these the bivalve *M. baltica* and the polychaete *Nereis diversicolor* occurred at all stations in all samples ($n = 44$). Species absent from the area concerned in 1965–71 (Fig. 5) and recolonizing it after 1972 include the amphipods *Pontoporeia affinis*, *Corophium volutator*, and *C. lacustre*, and the red midge larvae of the *Chironomus plumosus*-group. The oligochaete *Tubifex costatus*, the bivalve *Cardium* sp., and the polychaetes *Harmothoe sarsi* and *Polydora redeki* also colonized several of these localities in 1973 or 1974. These species are common at similar depths and bottom types of unpolluted parts of the Archipelago Sea with the exception of *Polydora*, which has been reported from only a few areas in southern Finland, close to intensively used ship channels.

The recolonization by benthos of the inshore sublittoral areas ceased by 1975. Signs of faunal deterioration were observed at three sampling sites (Stations 8, 9, and 10), nearest to the outflow from the new treatment plant. Not only the carnivorous midge larvae of the subfamily Tanypodinae, otherwise occurring at all the localities studied, but also *H. sarsi*, *Cardium* sp., and the bivalve *Mya arenaria* disappeared from these stations.

The faunal similarity between all macrobenthos samples taken ($n = 40$/yr) at 5–15 m deep (Stations 1–4 and 6–9), expressed as an index of percentage affinity between a set of samples (Sanders 1960), increased from 75 in 1972–73 to 89 in 1974–75. The latter pair of years yielded as many as 21 of the 23 species in common. In this depth zone, the dominance of crustaceans increased constantly from 0.2% of the total number of individuals in 1972 to 6.0% in 1975, and that of polychaetes from 5.5 to 8.0%. Simultaneously, the dominance value of Tanypodinae larvae decreased from 10.2 to 0.9% of the total fauna. The species that maintained their numerical dominance during the investigation period were *Macoma baltica*, *N. diversicolor*, *Peloscolex heterochaetus*, and the tubificid oligochaete *Euilyodrilus hammoniensis* (Fig. 6). In absolute numbers, however, the density of *M. baltica* decreased from the initial high values of ca. 3000 individuals/m² in this depth zone in 1973 to about 1400 individuals/m² in 1975. The biomass values of *M. baltica* remained unaltered or increased during this period and thus the average weight of clams doubled from approximately 50 to 100 mg/individual. This species is numerically the most important component of the bottom fauna of the northern Baltic archipelago waters. In the present material, for instance, it made up not less than 63% of the total number of benthic animals caught and 94% of the total measured biomass.

During the first years of recovery the bivalves *Mya arenaria* and *Cardium* sp. settled successfully at all inshore stations. In 1972 *M. arenaria* was sporadically found at only four stations (five individuals collected in total). In August 1974 young individuals (1–4 mm in shell length) of both these species occurred at all but one of the inshore sites (Fig. 4) in densities up to 10's or 100's of individuals/m². By 1975 the density

| 1972 1973 1974 1975 | AREA | BALLAST-WATER OUTFLOW | | NW BAY | | OIL HARBOR | | | PROCESS AREA | | TREATMENT PLANT | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ● ● ● ● | STATION | 1 | 2 | 3 | 4 | 5 | 6 | 47 | 7 | 8 | 9 | 10 |
| | DEPTH M | 6 | 11 | 14 | 12 | 16 | 13 | 21 | 7 | 10 | 6 | 3 |
| *Prostoma obscurum* | | ●--- | ---- | ---- | ---- | ---- | ---- | ---- | ---● | --●- | ●-●● | --●● |
| *Tubifex costatus* | | ---● | ---- | ---- | ●●●● | --●● | --●- | ●-●● | ---- | ---● | --●- | ---● |
| *Peloscolex heterochaetus* | | ●●●● | ●●●● | ●●●● | -●●● | ●-●- | ●●●- | ---● | --●- | ●●●● | ●●●● | ●-●● |
| *Euilyodrilus hammoniensis* | | ●●●● | ●●●● | ●●●● | ●-●● | ●-●- | ●●●● | ●-●● | --●- | ●●●● | ●●●● | --●- |
| *Limnodrilus hoffmeisteri* | | -●-● | -●-● | -●●● | ●-●- | --●● | ---- | --●● | --●● | -●-- | -●-- | ---● |
| *Nereis diversicolor* | | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● |
| *Harmothoe sarsi* | | ---● | ---- | -●●- | -●●● | --●● | --●● | ●-●● | --●- | -●●- | --●- | ---- |
| *Polydora redeki* | | -●●● | --●● | ---- | ●●●● | ●-●● | -●●● | ---- | --●● | ---● | --●● | --●● |
| *Manayunkia aestuarina* | | ---● | -●-- | ---● | ●-●- | --●- | ---- | ---- | --●- | ---- | --●- | ---- |
| *Pontoporeia affinis* | | --●- | --●● | --●● | ●-●● | ●●●● | ●-●● | --●● | ---- | ---- | ---- | ---- |
| *Gammarus spp.* | | -●-- | ●-●- | -●-- | ---- | -●-- | ---- | ---- | ●--- | ---- | ●●-- | --●- |
| *Corophium volutator* | | --●● | --●● | --●● | --●● | --●● | --●● | ---● | --●● | --●- | --●● | -●●- |
| *C. lacustre* | | --●● | --●● | ---- | ---● | ---● | ---- | ---- | --●- | ---● | ---- | ---- |
| *Mesidotea entomon* | | -●●● | --●- | ●●-● | ●-●● | ●●●● | ●●●● | ●●-● | ---- | --●- | ---- | --●- |
| *Chironominae spp.* | | ●●●- | ●--- | ---- | ---- | ---- | ●--- | ---- | ---- | ---- | -●-- | -●-- |
| *Chironomus plumosus* | | ---- | ---● | ---● | --●- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| *Tanypodinae spp.* | | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●- | ●●●- | ●●-- |
| *Potamopyrgus jenkinsi* | | -●●● | ●-●● | -●-- | -●-● | -●●● | ●●●● | ---- | -●-- | ---● | -●-● | --●● |
| *Hydrobia spp.* | | ●●-● | ●●●● | ●●●● | ●●●● | -●-● | ●●●● | ●●●- | ●●-● | -●●● | ●●●● | ●●●● |
| *Macoma baltica* | | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● | ●●●● |
| *Mya arenaria* | | ●●●● | ●-●● | -●●● | ●●●● | -●●● | ●●-● | -●-● | -●●● | --●- | --●- | --●● |
| *Cardium spp.* | | -●●● | -●●● | --●- | -●●● | --●● | --●● | -●-- | --●- | --●- | -●●- | --●● |
| *Mytilus edulis* | | -●-- | --●- | ---- | -●-● | ---- | ---- | ---- | ---- | ---● | ---- | ---- |

FIG. 4. Distribution of benthic species at the inshore sites in the vicinity of the oil refinery of Neste OY at Naantali in 1972–75. (●) = presence. (---) = absence. Tables showing the original materials are available as photocopies from the authors.

and the frequency of occurrence had decreased again but as a result of the growth of the few individuals left, the biomass values were considerably higher than one year earlier. The composition of the benthic fauna at Station 2 during 1972–75 is shown in Table 3 as a typical example.

At the inshore stations there was a very significant negative correlation ($-0.44^{xxx}$, $n = 50$; $t$-test) between the number of species per locality and the total oil content. This negative correlation was most conspicuous in 1972 and 1975, whereas the two years in between showed somewhat lower values.

## Bioassays with Chironomid Larvae

*Experiment 1* — When covered with oil-contaminated sediment, the larvae tended to avoid the substrate and some were found to move up to the sediment surface in the test dishes within a few hours (Fig. 7). In artificially polluted sediments with more than 0.75% oil the animals were inactivated or mechanically inhibited by oil, the burrowing activity decreased, and the larvae died.

*Experiment 2* — There was a clear correlation between the mortality of *Chironomus plumosus* and oil content in the artificially oil-contaminated sediments (Fig. 8). The topmost (0–2 cm) naturally contaminated sediment layer from the inshore stations off the refinery at Naantali caused a high mortality during a 28-d exposure in 1974 (Fig. 9). The lethal effect of these substrates upon the larvae decreased considerably by 1975 when 85% or more of the animals survived. Larval mortality in naturally oiled substrate from a deeper (about 10 cm) sediment layer from Station 7 remained unaltered. In sediments from Stations 2, 7, and 10, which were used for repeated biotests during 3 yr, the 50% mortality level was reached within the 1st wk of tests in 1973 and the exposure periods were not thereafter lengthened. Biotests made during 1974 and 1975 gave evidence of markedly improved sediment conditions at these three stations (Fig. 10).

*Chironomus plumosus* larvae appeared to be excellent laboratory animals: they are easily collected in great numbers and no mortality was observed for those larvae held in unpolluted control sediments during a 28-d exposure.

FIG. 5. Occurrence of the amphipods *Pontoporeia affinis* and *Corophium volutator* and red midge larvae (*Chironomus plumosus*-group) in the Naantali area in 1965–71 (according to Leppäkoski 1972, 1975a).

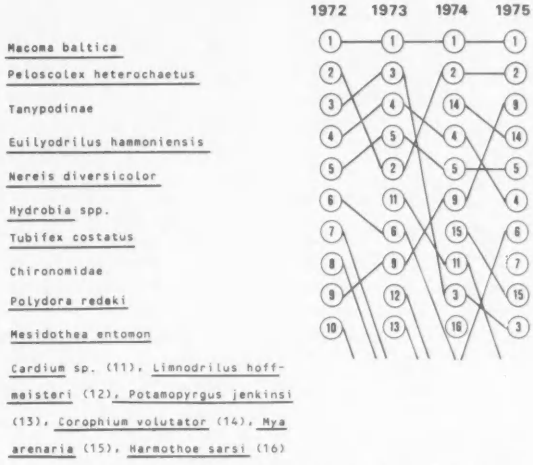


FIG. 6. Numerical dominance relationships of the 10 most dominant species (ranked after their percentage dominance of the total samples) in the 5–15-m depth zone at inshore Stations 1–4 and 6–9 in 1972–75.

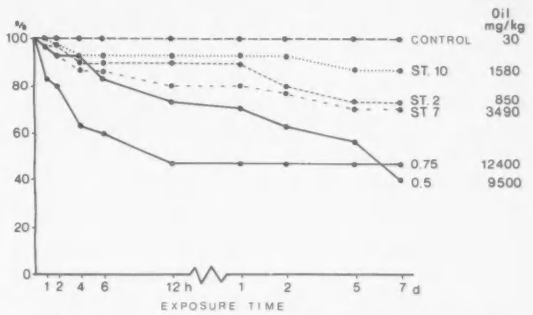


FIG. 7. Percentage of *Chironomus plumosus* larvae that remained in the sediment when covered with substrate taken from Stations 2, 7, and 10 or with artificially oil-contaminated substrate ($n = 3 \times 10$ larvae/substrate tested). The actual oil contents (mg/kg sediment dry weight) were measured by IR-spectrophotometry in September 1973.

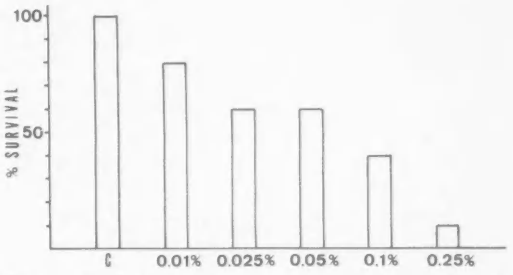


FIG. 8. Survival of *Chironomus plumosus* larvae in sediments contaminated with various percentages of oil during a 28-d exposure ($n = 4 \times 10$ larvae/substrate). C = control.

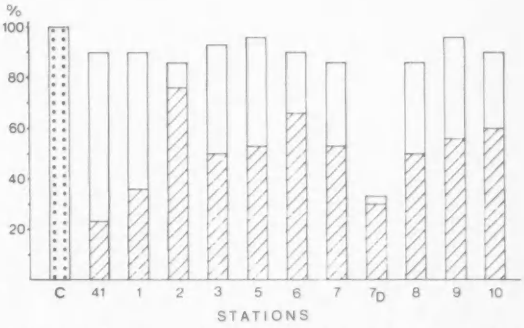


FIG. 9. Percent survival of *Chironomus plumosus* larvae in the topmost sediment layer (0–2 cm) taken from the inshore sites off the oil refinery of Neste OY at Naantali in 1974 (hatched) and 1975 (white; $n = 3 \times 10$ larvae/substrate). C = control. $7_D$ = deeper (about 10 cm) sediment layer from Station 7.

## Discussion

Accidental gross oil pollution often has serious or locally catastrophic effects on marine biota but in most cases these are reversible and temporary. The long-range sublethal effects of oils are quite a different matter, however. The effects of chronic oil pollution on the biota of sublittoral bottoms have been assessed quantitatively in very few cases. The rate of recovery from this type of pollution depends much on the rate and selective efficiency of oil biodegradation. Oil-degrading bacteria are present in low-temperature waters and degrade at least some of the fractions of oil, e.g. low molecular weight alkanes, cycloalkanes, and mono- and dinuclear aromatic hydrocarbons. By contrast, long-chain alkanes, polynuclear aromatic hydrocarbons, and asphaltenes are more resistant to biodegradation and little is known about the toxicity of the nondegradable end products (Cundell and Traxler

TABLE 3. Bottom fauna at Station 2 (11 m water depth) in 1972–75.

| | 1972 | | 1973 | | 1974 | | 1975 | |
|---|---|---|---|---|---|---|---|---|
| | ind/m² | g/m² | ind/m² | g/m² | ind/m² | g/m² | ind/m² | g/m² |
| *Peloscolex heterochaetus* | 326 | | 286 | | 1517 | | 1118 | |
| *Euilyodrilus hammoniensis* | 1641 | | 340 | | 390 | | 63 | |
| *Limnodrilus hoffmeisteri* | | | 46 | | | | 63 | |
| Tubificidae, total | 1976 | 1.38 | 672 | 0.47 | 1907 | 1.33 | 1244 | 0.99 |
| *Nereis diversicolor* | 107 | 5.88 | 55 | 2.51 | 80 | 1.84 | 50 | 2.05 |
| *Polydora redeki* | | | | | 189 | 0.08 | 758 | 0.50 |
| *Manayunkia aestuarina* | | | 4 | 0.01 | | | | |
| *Pontoporeia affinis* | | | | | 17 | 0.04 | 4 | 0.04 |
| *Gammarus* sp. | 4 | 0.01 | | | 34 | 0.04 | | |
| *Corophium volutator* | | | | | 17 | 0.08 | 163 | 0.21 |
| *C. lacustre* | | | | | 4 | 0.01 | 54 | 0.09 |
| *Mesidothea entomon* | | | | | 4 | 0.01 | | |
| Chironominae sp. | 13 | | | | | | | |
| *Chironomus plumosus* | | | | | | | 4 | |
| Tanypodinae | 751 | | 454 | | 88 | | 13 | |
| Chironomidae, total | 764 | 3.04 | 454 | 0.72 | 88 | 0.13 | 17 | 0.05 |
| *Hydrobia* spp. | 58 | 0.01 | 21 | 0.05 | 21 | 0.04 | 25 | 0.08 |
| *Potamopyrgus jenkinsi* | 13 | 0.01 | | | 17 | 0.04 | 8 | 0.04 |
| *Cardium* sp. | | | 8 | 0.01 | 25 | 0.01 | 4 | 0.01 |
| *Macoma baltica* | 3040 | 136.66 | 1684 | 187.05 | 2007 | 168.61 | 1462 | 163.28 |
| *Mya arenaria* | 4 | 0.01 | | | 50 | 0.04 | 46 | 14.75 |
| *Mytilus edulis* | | | | | 13 | 0.63 | | |
| Total | 5957 | 147.00 | 2898 | 190.81 | 4473 | 172.93 | 3835 | 182.09 |

1973; Hughes and McKenzie 1975). Growth of oil-degrading bacteria depends on temperature ($Q_{10}$ about 2–3) and the availability of oxygen and nutrients, especially nitrogen and phosphorus (e.g. Atlas and Bartha 1972; Boesch and Herschner 1974; Gibbs et al. 1975; Hughes and McKenzie 1975). In the Naantali area there are several municipal sewage outfalls; thus, organic enrichment may create favorable conditions for oil-degraders in the moderately polluted but still well-oxygenated sounds off the refinery.

Azoic or sparsely populated sediments are easily transported because of decreased cohesion. Thus, heavy oil contamination leads, by killing the sediment-living fauna, to washing out the deposited bottom material (cf. Blumer et al. 1971). The oil content will be expected to decrease if no longer continuously added. Recolonization or an increase in population density acts as a factor that tends to increase sediment cohesion. Simultaneously the increased biogenic reworking of the topmost sediment layers creates favorable conditions for an effective biodegradation of oil, as many of the species concerned (*Corophium*, *Polydora*, *Nereis*, Tubificidae, *Chironomus*) are tube dwellers and thus contribute to the aerating of the substrate down to depths of 5–20 cm below the surface. Otherwise, if no macrofauna are present, aerobic microbial activity takes place only in the uppermost millimetres of a sunken oil mass and oil degradation is thus very slow. Rates of degradation of oil in silt were estimated at 130–700 $g \cdot m^{-3} \cdot yr^{-1}$ by Hughes and McKenzie (1975), the rate being successively slowed as the most resistant fractions of oil are left. The oil spilled at West Falmouth (northeast coast of North America) in 1969 was still detectable in the sediments in 1973–74 but in significantly decreased amounts (Michael et al. 1975).

At Naantali, the bottom at the inshore stations down to 15 m deep consists mainly of firm clayey ooze or glacial clay, which gives evidence of rather slow sedimentation rate when compared with that of the outer intermediate sites, where very loose bottom materials dominate. In the inshore sublittoral zone the oil is thus apparently restricted to the youngest substrate layers and subjected to oxidation, dissolution, and microbial breakdown more effectively than the oils accumulated in the deeper soft-bottom areas off the refinery.

The first benthic survey made in the Naantali area in 1960 (Tulkki 1964), 3 yr after the refinery started, showed that most of the deeper localities below 20 m off the refinery and the oil harbor were azoic. These stations were colonized later but even in the late 60s the heavy oil pollution still caused bituminous odor in most of the sediment samples and clogged the sampling sieves. Unfortunately, there are no prepollution benthos data available. The effects of pollution on the bottom fauna were assessed in the present study in an indirect manner by (1) comparing the fauna near the refinery with that of nearby, less-contaminated or unaffected control sites, and by (2) monitoring the postpollution recovery, subsequent to a decrease in the amount of oil discharged.

Though a total of 23 taxa of sublittoral invertebrates

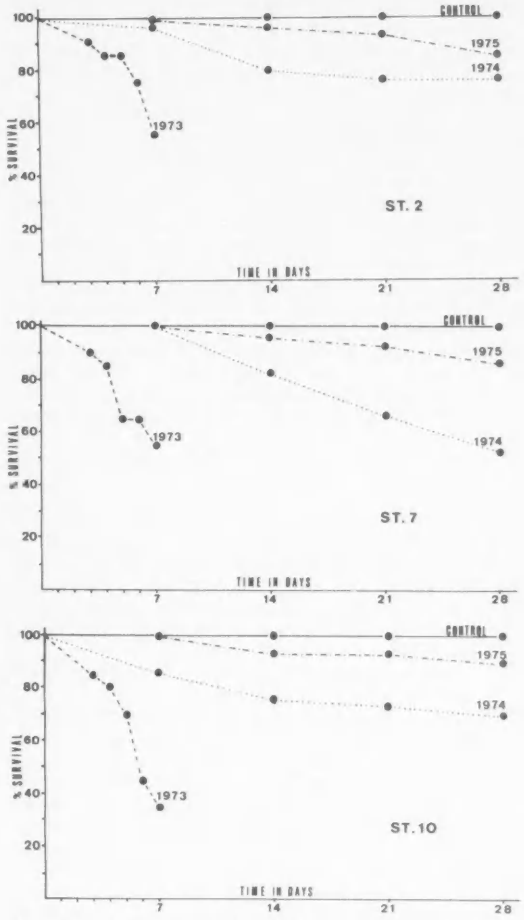


FIG. 10. Percent survival of *Chironomus plumosus* larvae in the 0–2-cm sediment surface layer from three inshore sites (Stations 2, 7, and 10) in 1973–75 ($n = 3 \times 10$ larvae/substrate).

was identified from the area, 95% of the benthic individuals of the deeper (below 20 m) near-refinery waters were of four species only (*Macoma baltica*, *N. diversicolor*, tanypodid midge larvae of the genus *Procladius*, and the tubificid oligochaete *E. hammoniensis*). *Macoma* and *Nereis* are known to be highly resistant to oil pollution and to refinery effluents (König 1968; Baker 1973; Kasymov and Aliev 1973; cf. also Shaw et al. 1976). Due to the insensitivity of these biomass dominants, rather high standing crop levels were maintained even in the numerically and structurally most impoverished benthic assemblages.

The species that were absent from the inshore area prior to 1973–74 but lived in nearby, less contaminated waters include animals that may be mechanically affected by oil in the substrate. One such species, the red midge larva (*C. plumosus*-group) has failed to appear in the oil-contaminated area in Naantali for at least 15 yr but was found at some stations in 1974–75. These larvae are not able to build their tubes in oil-polluted lake sediments and lethal effects may be observed at low contamination levels (Bengtsson and Berggren 1972; Kasymov and Aliev 1973), as was also shown by our laboratory experiments. Dispersed oil may also affect the suspension feeding habits typical of chironomid larvae living in U-shaped tubes in deeper waters (McLachlan 1977).

*Chironomus plumosus*-type larvae have proved to be highly sensitive "indicators" for contaminants other than oil in low-salinity brackish-water areas (Leppäkoski 1975a), whereas many other indicator species are the last survivors in oil-contaminated substrates. These latter species are not necessarily the most oil-tolerant species in laboratory experiments but survive better in field conditions when exposed to complex pollution stresses (cf. Moore and Dwyer 1974; Rossi et al. 1976). Burrowing activity of tube dwellers, many of them successful recolonizers in the study area after pollution abatement, may be hindered by oil and as a result the animals are subject to predation (Wharfe 1975). The postabatement recolonizors found in the vicinity of the Neste OY refinery at Naantali were mainly marine and brackish-water species, which are sensitive indicators of healthy bottoms in the inner parts of the Archipelago Sea (Tulkki 1969; Leppäkoski 1975a).

Significant decreases in benthic diversity in oil-contaminated water and increasing values in the zone of recovery have been reported previously (Wilhm and Dorris 1968; Michael et al. 1975). However, the remarkable increase in benthic diversity observed in several investigations during the first years of succession after pollution abatement (Reish 1971, 1973; Rosenberg 1973, 1976; Leppäkoski 1975a; Michael et al. 1975, as well as the present study) may be temporary and artificial.

Postpollution and other postdisturbance communities consist not only of the most resistant forms left from the former disclimax community but also of faunal opportunists (cf. Grassle and Sanders 1973; Grassle and Grassle 1974; Leppäkoski 1975b; Michael et al. 1975) as well as representatives of the new, stabilizing community. Each of these groups may influence in part the first intensive resettlement of formerly azoic or greatly impoverished areas as well as the maturation of the benthic community in dynamic equilibrium with the surrounding environment. As shown by Grassle and Grassle (1974) and Rosenberg (1976), any studies of recovery from pollution in aquatic ecosystems should be extended to include not only the initial seral stages of succession but also the later stages, in which density-dependent factors and interactions within the community regulate the biologically controlled populations of species with more specialized niches than those of

the opportunists. The time needed for benthic recovery from accidental or chronic oil pollution has been variously estimated at 2–20 yr (Blumer et al. 1971; Reish 1971, 1973; Bugbee and Walter 1973; Meynell 1973; Moore and Dwyer 1974; Boesch and Herschner 1974; Wharfe 1975). However, in most cases the level of recovery referred to has remained undefined.

In the present study, laboratory experiments gave evidence of markedly improved sediment conditions for one of the species involved during the 1st yr of recovery. During the same time changes in macrobenthic fauna at the inshore sites indicated generally better conditions for a variety of benthic animals. By 1975, however, some of these changes appeared to be reversing, the faunal assemblages resembling more and more those of the organically enriched sounds off the nearby towns where the macrobenthic fauna is characterized by the overwhelming dominance of *M. baltica* (the biomass values of which exceed several times those of unaffected coastal areas in southwest Finland) *N. diversicolor*, tubificid oligochaetes, and chironomid larvae (Leppäkoski 1975a). This trend continued in 1976 (T. Juuti personal communication). Thus, an investigation period of 3 or 4 yr is not long enough for monitoring the final stages of a postabatement succession.

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# Recruitment of Benthic Animals as a Function of Petroleum Hydrocarbon Concentrations in the Sediment[1]

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Three separate field installations, consisting of clean and oiled sediment in fiberglass trays, were placed in the intertidal zone of Sequim Bay, Washington, to determine rates of hydrocarbon depuration and recruitment of benthic organisms. Detailed chemical analysis, with glass capillary gas chromatography and gas chromatography–mass spectroscopy, were conducted such that individual compounds and hydrocarbon classes associated with the sediment after varying periods of field depuration could be quantitated. Depuration rates of hydrocarbon types in sediment receiving oil on the surface (installations I and II) decreased in the order of saturates, methylnaphthalenes, and methylphenanthrenes. Rates of specific compound and hydrocarbon class depuration followed the general pattern exhibited by total hydrocarbons (infrared analyses). In a period of 100 d, total hydrocarbons in surface-oiled, coarse sediments (I and II) decreased by 82–88%, while the amount in the finer substrate with mixed in oil (III) only decreased by about 21% and remained quite stable up to 290 d. The ratios of $n$C$_{17}$/pristane and $n$C$_{18}$/phytane were monitored for installations I and III (oil mixed with sediment). The ratios remained constant for a period of 4–6 mo and then dropped sharply, suggesting the presence of oil biodegradation. These results are discussed in relation to physical and chemical processes acting on the oil-contaminated sediments. Initial concentrations of oil in sediments upon field emplacement were about 5000–6000 ppm in installations I and II and approximately 700 ppm in installation III. At these concentrations, no substantial inhibition of recruitment by benthic organisms was observed. Future sampling of these populations and further analyses of all benthic organisms may provide a better evaluation of effects of specific hydrocarbon components in sediments on benthic recruitment. These results are discussed in light of oil spill studies and other field experiments.

*Key words:* benthic organisms, gas chromatography, hydrocarbon, petroleum, sediment, spectroscopic techniques

ANDERSON, J. W., R. G. RILEY, AND R. M. BEAN. 1978. Recruitment of benthic animals as a function of petroleum hydrocarbon concentrations in the sediment. J. Fish. Res. Board Can. 35: 776–790.

Trois installations séparées sur le terrain, constituées de plateaux de fibre de verre contenant un sédiment propre et un sédiment imbibé de pétrole, ont été placées dans la zone intertidale de la baie Sequim, Washington, dans le but de déterminer les taux d'épuration des hydrocarbures et de recrutement d'organismes benthiques. Les auteurs ont fait une analyse chimique détaillée, par chromatographie gazeuse sur capillaire de verre et chromatographie gazeuse/spectroscopie de masse, de façon à pouvoir associer avec le sédiment les composés individuels et les classes d'hydrocarbures après avoit quantifié différentes périodes d'épuration sur le terrain. Les taux d'épuration des types d'hydrocarbures dans le sédiment recevant du pétrole à la surface (installations I et II) diminuent dans le même ordre que les produits de saturation, les méthylnaphtalènes et les méthylphénanthrènes. Les taux d'épuration de composés spécifiques et de classes d'hydrocarbures sont en général les mêmes que ceux des hydrocarbures totaux (analyses aux infrarouges).

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Au cours d'une période de 100 jours, les hydrocarbures dans les sédiments grossiers recevant du pétrole à la surface (I et II) diminuent de 82 à 88%, alors que dans le substrat plus fin mélangé de pétrole (III), la quantité d'hydrocarbures ne diminue que de 21% et demeure à peu près stable durant une période allant jusqu'à 290 jours. Les auteurs ont suivi de façon continue les rapports $nC_{17}$/pristane et $nC_{18}$/phytane aux installations I et II (pétrole mélangé au sédiment). Les rapports demeurèrent uniformes durant une période de 4–6% mo, pour ensuite baisser brusquement, laissant croire à une biodégradation du pétrole. On analyse ces résultats par rapport aux processus physiques et chimiques agissant sur les sédiments contaminés par le pétrole. Les concentrations initiales de pétrole dans les sédiments aux installations sur le terrain sont d'environ 5 000 à 6 000 ppm aux installations I et II et approximativement 700 ppm à l'installation III. Les auteurs n'ont pas observé d'inhibition substantielle dans le recrutement des organismes benthiques à ces concentrations. On sera mieux à même d'évaluer les effets d'hydrocarbures spécifiques dans les sédiments sur le recrutement des organismes benthiques en poursuivant l'échantillonnage de ces populations et d'autres analyses de tous les organismes benthiques. On examine ces résultats en fonction d'études de déversements accidentels de pétrole et autres expériences sur le terrain.

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As a result of oil production from the Alaskan outer continental shelf, the Pacific Northwest is one of the areas of the United States most likely to receive increased inputs of petroleum hydrocarbons from transport, port operations, and refining. There is the possibility that exploration on the coasts of Oregon and Washington will indicate the potential for drilling, and thus, production-related inputs are possible. Present and future activities associated with this energy source necessitate research on the potential impacts of oil in the Pacific Northwest marine environment. While the results of recent studies on the fate and effects of petroleum hydrocarbons in the marine environment have strengthened our understanding of some aspects of oil pollution, we are not yet to the point at which an accurate prediction of the effects of acute or chronic oil inputs can be made.

The experimental approach utilized in this study was designed to provide correlations between the rates of oil depuration and benthic organism recruitment in the natural environment. This type of field experiment has been recommended in past workshops (Beynon and Cowell 1974; EXXON 1974), but to our knowledge has not yet been conducted. It is most important to emphasize the need for quantitative data on the specific components of oil that impact organisms and the duration of their presence in the marine environment. Very few field studies have incorporated detailed chemical analyses with measurements of effects on organisms. Lee and Anderson (1977) described the fate and effects of naphthalenes in an experimental field enclosure, but only the water column was monitored. Shaw et al. (1976) conducted field exposures up to 44 d to oiled substrate using the clam *Macoma baltica* and showed an effect of surface oiling on mortality. Application of 5.0 $\mu$L oil/cm$^2$ produced significant mortality, and total oil concentrations (gravimetrically determined) in final sediment samples ranged from 760 to 3890 ppm (dry weight). Because this animal is a deposit feeder, it is expected that surface oiling would result in high contamination of tissue and mortality. Uptake studies with the same sediment trays described in this paper have shown the bioavailability of specific petroleum hydrocarbons to *M. inquinata* (Roesijadi et al. 1978a, b).

A report by Armstrong et al. (1977) provides information on the effects of chronic hydrocarbon exposure on a benthic community. A separator platform effluent in Galveston Bay, Tex., introduced hydrocarbons into a 3-m water column at levels of about 25 ppm, of which approximately one half of the components were aromatics. The water contained only about 10 ppb aromatics, while sediment beneath the platform contained a total of 96 ppm, of which 34 ppm were aromatics. The aromatics measured were primarily naphthalenes, biphenyls, fluorenes, and phenanthrenes. The concentrations in this fine clay substrate resulted in reduced numbers of species and individuals of benthic organisms living within 152 m of the platform. The abundance of organisms over an 18-mo period was correlated with naphthalene content along three transects.

Bieri and Stamoudis (1977) reported on the results of a No. 2 fuel oil spill experiment in a small area of a marsh. While significant levels of hydrocarbons were found in the water column (quite shallow) and clam and oyster tissue, no spill-related hydrocarbons were found in extracts of the sediment. The analytical techniques used by these investigators were quite sensitive, but background levels in the sediment of the area were relatively high. MacLeod et al. (1977) reported high sediment hydrocarbon levels in samples collected near a creek that had received fuel oil from a leaking storage tank. These investigators also used very sensitive chemical methods to show the dramatic differences between concentrations of various oil components in the contaminated creek area (Port Angeles, Wash.) and a relatively pristine area (Dungeness Bay, Wash.). They prepared a list of compounds worthy of use in monitoring hydrocarbon contamination that included naphthalenes, phenanthrenes, fluoranthrene, chrysene, pyrene, benzo[*a*]pyrene, and others. Concentrations in the polluted sediment of phenanthrene, fluoranthrene,

and pyrene were generally on the order of 200–1000 ng/g dry weight (ppb).

The objective of this investigation was to define closely the initial and subsequent levels of specific petroleum hydrocarbon components in contaminated substrate placed in the natural environment and examine their influence on benthic recruitment. This approach would provide data on the depuration rates of specific hydrocarbons and classes of compounds under natural intertidal conditions, and allow correlation of contamination characteristics with any effects on the settlement and survival of benthic species. The research is continuing such that alterations in the biota and chemical characteristics of the sediment may be followed for a period of years to provide realistic information on the fate and effects of petroleum hydrocarbons in a cold-water marine sediment. Because the chemical data were obtained earlier and comprehensive analyses of chemical and biological data are beyond the scope of a single publication, this paper encompasses the details of hydrocarbon alterations and a summary of benthic recruitment. The distribution of species within the sediment trays will be treated in detail, with various statistical analyses, in a later manuscript.

## Materials and Methods

### Analytical Chemistry

*Separation and quantitation of compounds in contaminated sediment* — Triplicate 20-g samples of sediment were extracted for 24 h in glass bottles (having Teflon-lined caps) containing 20 g of anhydrous sodium sulfate and 50 mL of hexane (Burdick & Jackson). Appropriate amounts, based on total oil analysis of each sample, were concentrated to one mL and separated into saturate and aromatic fractions by silica-gel chromatography, described below. Saturate and aromatic fractions were analyzed by capillary column chromatography, with 2,6,10-trimethyldodecane and hexamethylbenzene as internal standards for the saturate and aromatic fractions, respectively. Peak areas and internal standard calibrations were determined with an Autolab System IV computer integrator. Final concentrations were expressed in terms of wet weight of sediment.

*Identification and quantitation of saturate and aromatic hydrocarbons in Prudhoe Bay crude (PBC) oil* — The separation of PBC into a saturate and aromatic fraction was adapted from a silica-gel chromatography technique described by Warner (1976) and modified by MacLeod et al. (1977). Each fraction with its appropriate internal standard was concentrated and analyzed by gas chromatography on a 30-m SE-30 glass capillary column (J & W Scientific) programmed from an initial temperature of 70°C to 250°C at a rate of 4°C/min. Compounds in the saturate and aromatic fractions were identified by peak-enhancement analysis with known standards. Identification and quantitation of compounds in the aromatic fraction proved to be more difficult because of the lack of availability of commercial standards and because many were present in PBC at much lower concentrations relative to the saturate compounds.

A Hewlett Packard 5982A quadrapole mass spectrometer operating in the argon chemical ionization mode and employing single-ion monitoring was used to determine approximate retention time ranges for specific compound types within the aromatic fraction. These compound types included monomethyl-, dimethyl-, and trimethylnaphthalenes, monomethyl-, $C_2$-, and $C_3$-phenanthrenes, etc. The GC–MS information has thus enabled us to estimate the concentration of hydrocarbon types by calculating the total concentrations on the basis of those compounds that reside within a given retention time range. Compounds and compound classes identified in the saturate and aromatic fractions are listed in Fig. 1 and 2.

*Recovery studies* — To determine the efficiency of extraction of hydrocarbons from the oiled sediment, recovery studies with hexane as the solvent were conducted. One millilitre of two concentrations of oil in hexane was added to separate 20-g samples of sediment. The concentrations of oil chosen approximated the extremes of oil concentration range observed in the sediment field depuration studies. Each sample was extracted, concentrated on the basis of total oil concentration, and analyzed as previously described. Percent recoveries of individual compounds and compound types isolated from the oil were calculated on the basis of the known amount of oil added to the sediment and the concentrations of individual compounds present in PBC. Average recoveries of saturate hydrocarbons from sediment for both concentration levels ranged from 83 to 85%. Similar results were obtained for naphthalene (100%), methylnaphthalenes (88–99%), phenanthrene (80–96%), and methylphenanthrenes (84–90%).

*Analysis of total oil in sediments by IR* — Concentrations of total oil in sediments were determined on $CCl_4$ extracts of sediments with a Beckman Acculab 6 spectrophotometer by comparison of the amount of absorbance obtained at $2930 \cdot cm^{-1}$ to a calibration curve prepared with Prudhoe Bay crude oil (American Petroleum Institute 1958). Recoveries of total oil from $CCl_4$ extracts of oil-amended sediments were found to be >90%.

### Biological Procedures

*Experimental system* — The methods and materials used in these studies are essentially those described for oil-on-sediment and oil-in-sediment exposures conducted with sipunculids (*Phascolosoma*) (Anderson et al. 1977). Either fiberglass or polyvinylchloride (PVC) sediment trays (36 × 46 × 81 cm), containing three equal compartments and fiberglass mesh (1.6-mm openings) in the bottom, were used to hold clean and contaminated sediment in the intertidal zone. Sediment was collected from the same area that was used for placement of experimental trays. Sieving of natural substrate was necessary to reduce large variability in particle size, ranging from fine detritus to large cobbles. The first two installations (I on April 17, and II on May 3, 1976) consisted of six trays each, four of which received oil while two served as controls. The sediment used in these installations was sieved through a 12-mm mesh screen in the field and transferred in buckets to a freezer at the laboratory. After three cycles of freezing and thawing, the substrate was placed on the trays and transferred to large aquaria receiving flowing seawater (1 L/min). The aquaria were equipped with a system to provide tidal flushes such that water could drain completely from the sediment

TABLE 1. Sampling schedule for field installations. Trays (T) 4 and 5 contained control sediment in all installations. There were three sections per tray and one was completely emptied at each time interval. Numbers in parentheses are the number of replicate cores sampled.

| Condition | Dates of sampling trays and sections | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1976 | | | | | | | | | 1977 | | | | | | | |
| | A | M | J | J | A | S | O | N | D | J | F | M | A | M | J | J | A |
| *Installation I* | | | | | | | | | | | | | | | | | |
| Coarse sediment (12-mm sieve) oil on top, flushed 48 h in lab | 4/17[a] T 1, 3, 5 Sec. 1 (3) | | 6/21 T 2, 4, 6 Sec. 1 (3)[b] | | 8/24 T 1, 3, 5 Sec. 3 (3)[b] | | | 11/23 T 2, 4 Sec. 3 (3)[c] | | 2/15 T 1, 3, 5 Sec. 2 (5) | | | | (Term.) 5/6 T 2, 4, 6 Sec. 2 (5) | | | |
| *Installation II* | | | | | | | | | | | | | | | | | |
| (As I above) | | 5/3 T 1, 3 Sec. 1 (3) | | | 8/24 T 2, 4, 6 Sec. 1 (3)[b] | | | 11/23 T 1, 3, 5 Sec. 3 (3)[c] | | 2/15 T 2, 4, 6 Sec. 3 (5) | | | | 5/6 T 1, 3, 5 Sec. 2 (5) | | | 8/14 T 2, 4, 6 Sec. 2 (5) |
| *Installation III* | | | | | | | | | | | | | | | | | |
| Fine sediment (2-mm sieve) oil mixed in, flushed 48 h in lab | | | | | | | 10/28 T 1, 3, 5 Sec. 1 (3) | | | 2/15 T 2, 4, 6 Sec. 1 (5) | | | | 5/6 T 1, 3, 5 Sec. 3 (5) | | | 8/14 T 2, 4, 6 Sec. 3 (5) |

[a]Month/day.
[b]After extraction of two cores for chemical analyses all the remaining sediment in the section was sorted for organisms.
[c]As above, except the remaining sediment was mixed and four subsample cores were taken in the laboratory.

(through the mesh bottom) and fill with aerated water after a "low tide" period of 1 h. When the water level was approximately 1 cm above the sediment surface, a 4% (vol/vol) volume of oil was poured over the water, forming a uniform layer. "Low tide" was then initiated, and the oil impacted the surface and remained there for about 2 h, until the next "high tide." At the high water level, excess oil (not adsorbed to sediment) was skimmed off by the outflow tube. After two more tides (48 h total), the four exposed trays and two controls were transferred to the zero tide level on the sand spit east of the laboratory. Exactly the same procedure was used in the preparation of installation II in May 1976.

To simulate a spill, where finer sediment is thoroughly mixed with oil, installation III (October, 1976) was prepared in a somewhat different manner. Sediment collected from the same locale was sieved a second time to eliminate particles >2 mm. As described in the oil-in-sediment procedure of Anderson et al. (1977), this substrate was mixed thoroughly in a fiberglass-lined cement mixer with a volume of oil approximately 0.1% of the sediment volume. The oil was mixed in a blender with 500 mL of seawater before addition to the tumbling moist sediment, and a 500-mL rinse was added a few moments later. Using a $C^{11}$-labeled-2 methylnaphthalene spike in the oil, Anderson et al. (1977) have found that periods of mixing between 15 min and 1 h are sufficient to produce oil–sediment homogeneity. These trays were also flushed for 48 h in the laboratory tidal-flux system to remove excess oil. They were placed in the field near the first two installations on October 28, 1976.

*Sampling and analyses* — The schedule of sampling is shown in Table 1. With the initiation of installation I as an example, on April 17, 1976, after a 48-h flushing period in the laboratory, three cores were taken from section 1 of trays 1, 3, and 5, and frozen for later analyses. Since organisms had been killed previously by freezing and thawing, no samples were taken for biota analysis at initiation of each set of installations (April 17, May 3, and October 28, 1976). The cores were sections of standard PVC pipe measuring about 13.2 cm long, with an ID of 3.8 cm. The mean volumes and standard deviations of sediment extracted by cores were 93 ± 14 mL (I and II) and 97 ± 12 ml (III). An individual core represented about 5% of a tray section, which contained approximately 2 L of sediment. Small differences among installations were the result of minor sediment height variations. In the laboratory and in the field, core samples were easily removed by inserting all replicates (usually five) and placing a neoprene stopper over one at a time before slowly extracting the tube. The contents of three or more cores were allowed to extrude into widemouthed jars and within 2 h 5% Formalin was added to each. After about 1 wk the fixative was carefully decanted, and a 70% ethanol solution containing Rose Bengal stain was added to aid in distinguishing between organisms and detritus particles. Other replicate cores for chemical analyses were, within 1 h, placed in a low-temperature (−70°C) freezer until transport on dry ice and subsequent analyses.

Somewhat different techniques were used for early field collections on June 21, August 24, and November 23, 1976. Two core samples were taken as already described and

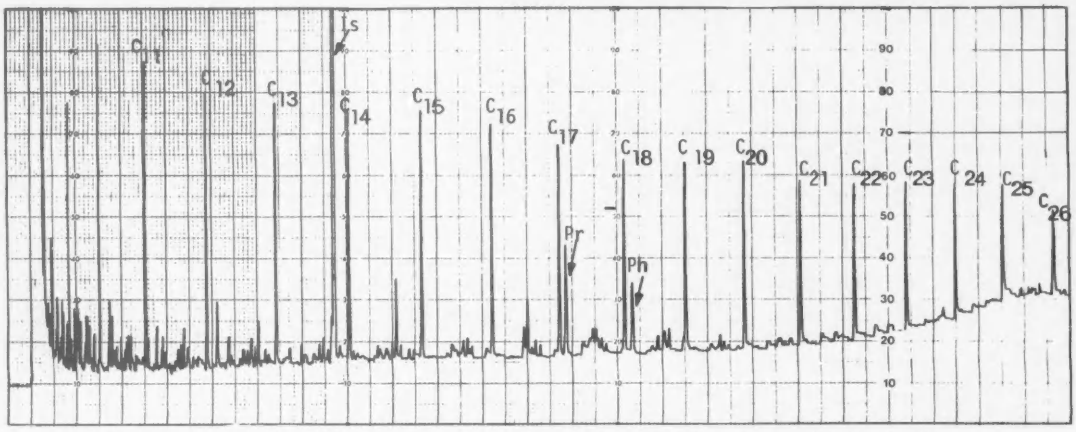



FIG. 1. Gas capillary chromatogram of saturate fraction of Prudhoe Bay crude oil: $C_{11} \rightarrow C_{26}$, saturate straight-chain hydrocarbons, Pr = pristane, Ph = phytane, Is = 2,6,10-trimethyldodecane internal standard.

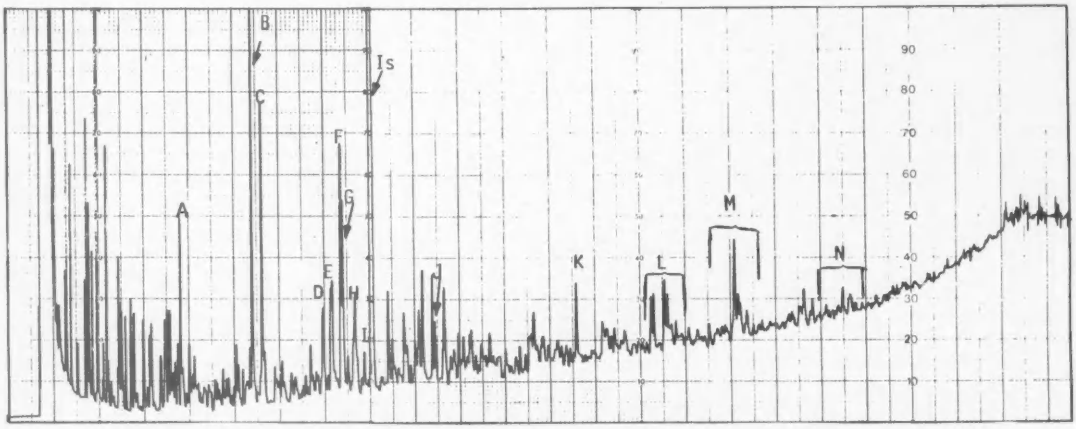



FIG. 2. Gas capillary chromatogram of aromatic fraction of Prudhoe Bay crude oil: A. naphthalene; B. 2-methylnaphthalene; C. 1-methylnaphthalene; D. 1-ethyl + 2-ethylnaphthalene; E. 2,6 + 2,7*-dimethylnaphthalene; F. 1,3 + 1,6-dimethylnaphthalene; G. 1,7*-dimethylnaphthalene; H. 1,4 + 2,3 + 1,5-dimethylnaphthalene; I. 1,2-dimethylnaphthalene; J. 2,3,6-trimethylnaphthalene; K. phenanthrene; L. methylphenanthrenes; M. $C_2$-phenanthrenes; N. $C_3$-phenanthrenes; Is = hexamethylbenzene internal standard. (*2,7 and 1,7 are suggested structures based on published literature on the low-resolution gas chromatographic separation of dimethylnaphthalenes.)

frozen for chemical analyses, but for the June and August collections, all the remaining sediment in the section was sorted for organisms. This process was clearly too labor intensive, so subsample cores were taken from the November collection, which were comparable to those taken in the field at all later dates.

Sorting of organisms from small portions (about 5 mL) of the sediment was conducted under a dissecting microscope at 15×. Animals were placed in small vials with 70% ethanol and held for later identification and counting. While a large number of species were identified, the more prominent species which were present at nearly every interval and in each sample were selected for comparisons.

## Results

### CHEMICAL

*Analysis of Prudhoe Bay crude* — Compounds identified in the saturate fraction of PBC by gas capillary column chromatography included the straight-chain hydrocarbons from $C_{11}$ to $C_{26}$, pristane, and phytane.

TABLE 2. Concentrations of saturate and aromatic hydrocarbons (parts per million, ppm) in sediment from the day of installation of field experiment I (April 17, 1976). MN = Methylnaphthalene, DMN = Dimethylnaphthalene, TMN = Trimethylnapthalene, MP = Methylphenanthrene.

| Saturates | Concn (ppm) | Aromatics | Concn (ppm) |
|---|---|---|---|
| $C_{11}$ | 13.34±1.21 | Naphthalene | 7.20±0.77 |
| $C_{12}$ | 16.11±1.53 | | |
| $C_{13}$ | 17.79±1.82 | 2-MN | 14.31±3.10 |
| $C_{14}$ | 17.44±2.39 | 1-MN | 10.51±2.90 |
| $C_{15}$ | 19.39±2.94 | Total | 24.82±5.32 |
| $C_{16}$ | 18.84±3.00 | | |
| $C_{17}$ | 17.03±2.92 | 1-ethyl + 2-ethyl | |
| Pristane | 10.74±1.95 | naphthalene | 6.93±0.35 |
| $C_{18}$ | 16.20±3.22 | | |
| Phytane | 8.05±1.43 | 2,6 + 2,6-DMN | 9.52±0.75 |
| $C_{19}$ | 18.79±3.00 | 1,3 + 1,6-DMN | 9.93±0.94 |
| $C_{20}$ | 16.40±2.88 | 1,7-DMN | 7.81±0.64 |
| $C_{21}$ | 17.56±3.09 | 1,4 + 2,3 + 1,5 DMN | 9.14±0.72 |
| $C_{22}$ | 16.02±3.37 | 1,2-DMN | 1.60±0.45 |
| $C_{23}$ | 15.18±2.65 | Total | 42.68±2.74 |
| $C_{24}$ | 13.80±2.84 | | |
| $C_{25}$ | 11.71±2.47 | TMN-1 | 3.80±0.64 |
| $C_{26}$ | 10.21±1.97 | TMN-2 | 3.65±0.55 |
| | | TMN-3 | 5.69±1.11 |
| Total saturates | | TMN-4 | 4.26±0.76 |
| measured | 274.61±42.32 | 2,3,6-TMN | 2.08±0.24 |
| | | TMN-5 | 5.99±0.77 |
| | | Total | 28.44±6.10 |
| | | Phenanthrene | 3.05±0.85 |
| | | MP-1 | 2.74±0.56 |
| | | MP-2 | 2.34±0.40 |
| | | MP-3 | 3.94±1.71 |
| | | MP-4 | 3.49±1.67 |
| | | Total | 12.53±4.31 |
| | | $C_2$-phenanthrene-1 | 7.30±1.56 |
| | | $C_2$-phenanthrene-2 | 2.36±0.68 |
| | | $C_2$-phenanthrene-3 | 2.56±0.44 |
| | | $C_2$-phenanthrene-4 | 2.47±0.87 |
| | | Total | 14.70±3.52 |
| | | Total aromatics measured | 140.35±23.96 |

Pristane and phytane were baseline resolved from heptadecane and octadecane (Fig. 1). In the aromatic fraction, naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, several of the dimethylnaphthalenes, 2,3,6-trimethylnaphthalene, and phenanthrene were identified. Other aromatic compounds, although not specifically identified, were shown to be members of a specific compound class (Fig. 2).

*Sediment analyses* — The above method was applied to samples of intertidal sediment in the field for various periods, to determine the depuration rates of components of crude oil. The concentrations of compounds in the saturate and aromatic fractions from sediment taken from the 1st d of the first installation are listed in Table 2. Docosane, naphthalene, and phenanthrene were selected as representative saturate and aromatic components of oil, and their rate of depuration in the sediment was determined over a period of 7 mo (Fig. 3). Only 2.1, 1.0, and 2.6% of the original docosane, naphthalene, and phenanthrene, respectively, remained in the surface portion of the sediment after 7 mo of exposure in the beach environment. Similar trends were found for three representative compound classes, as only 1.8, 1.1, and 4.0% of the saturates, methylnaphthalenes, and methylphenanthrenes remained after the same period of time (Fig. 4). These results were in excellent agreement with the observed depuration according to total oil analysis obtained on the same samples (Fig. 4).

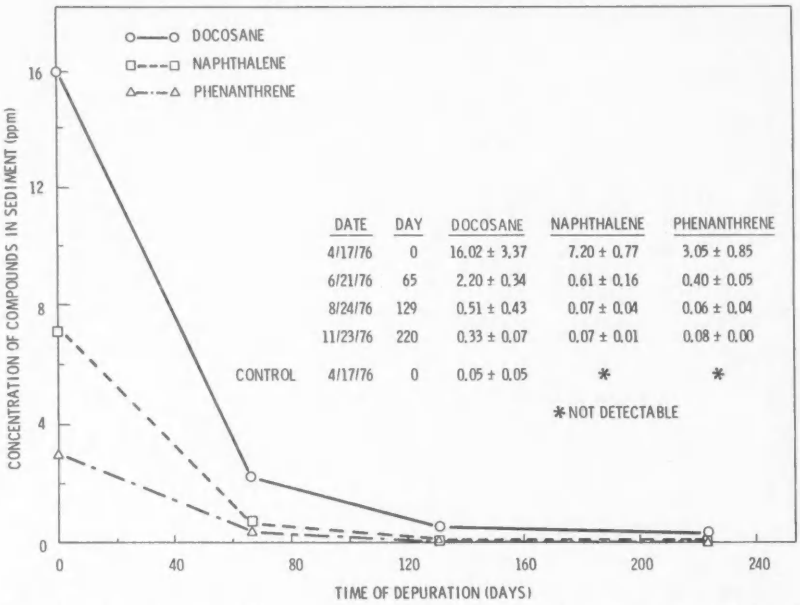

DOCOSANE
NAPHTHALENE
PHENANTHRENE
CONCENTRATION OF COMPOUNDS IN SEDIMENT (ppm)
TIME OF DEPURATION (DAYS)
DATE DAY DOCOSANE NAPHTHALENE PHENANTHRENE
4/17/76 0 16.02 ± 3.37 7.20 ± 0.77 3.05 ± 0.85
6/21/76 65 2.20 ± 0.34 0.61 ± 0.16 0.40 ± 0.05
8/24/76 129 0.51 ± 0.43 0.07 ± 0.04 0.06 ± 0.04
11/23/76 220 0.33 ± 0.07 0.07 ± 0.01 0.08 ± 0.00
CONTROL 4/17/76 0 0.05 ± 0.05 * *
*NOT DETECTABLE

FIG. 3. Concentrations of docosane, naphthalene, and phenanthrene in sediment of installation I, as a function of depuration time.

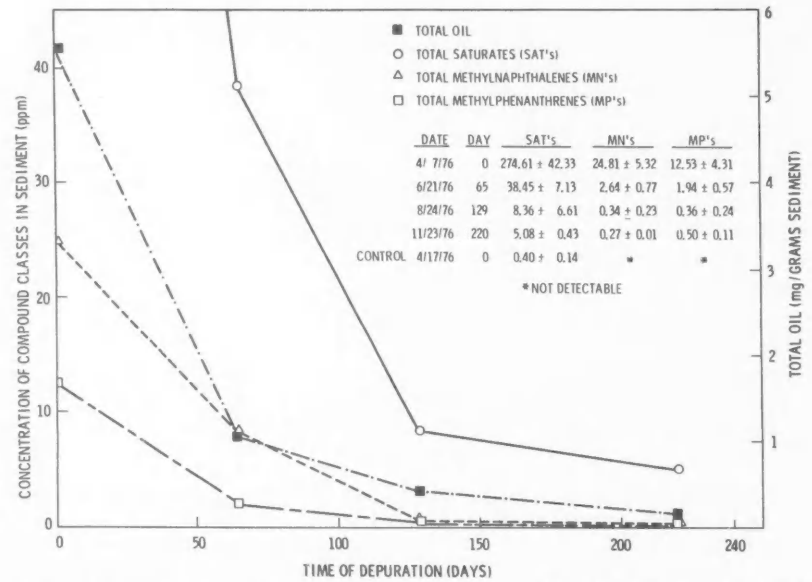

TOTAL OIL
TOTAL SATURATES (SAT's)
TOTAL METHYLNAPHTHALENES (MN's)
TOTAL METHYLPHENANTHRENES (MP's)
CONCENTRATION OF COMPOUND CLASSES IN SEDIMENT (ppm)
TOTAL OIL (mg/GRAMS SEDIMENT)
TIME OF DEPURATION (DAYS)
DATE DAY SAT's MN's MP's
4/ 7/76 0 274.61 ± 42.33 24.81 ± 5.32 12.53 ± 4.31
6/21/76 65 38.45 ± 7.13 2.64 ± 0.77 1.94 ± 0.57
8/24/76 129 8.36 ± 6.61 0.34 ± 0.23 0.36 ± 0.24
11/23/76 220 5.08 ± 0.43 0.27 ± 0.01 0.50 ± 0.11
CONTROL 4/17/76 0 0.40 ± 0.14 * *
*NOT DETECTABLE

FIG. 4. Concentrations of compound classes in sediments of installation I as a function of depuration time.

The rates of depuration of these compounds and compound types in the second installation were very similar to the rates observed in the first field installation. Initial oil concentrations were approximately the same. After a 7-mo time period, only trace amounts of docosane remained, and naphthalene and phenanthrene

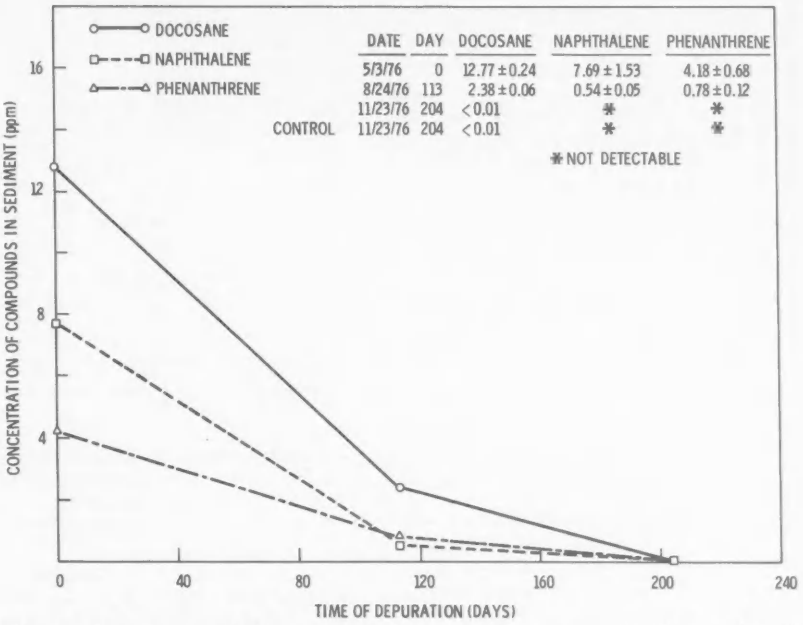



FIG. 5. Concentrations of docosane, naphthalene, and phenanthrene in sediment of installation II, as a function of depuration time.

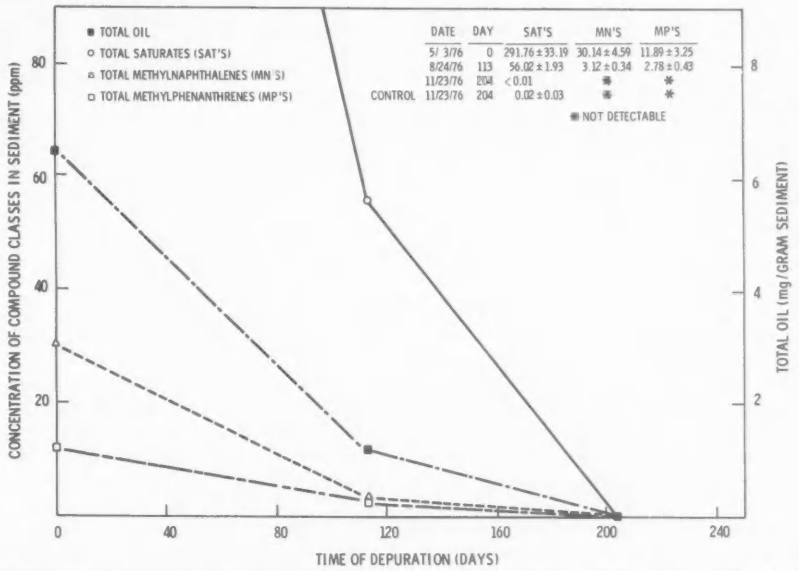
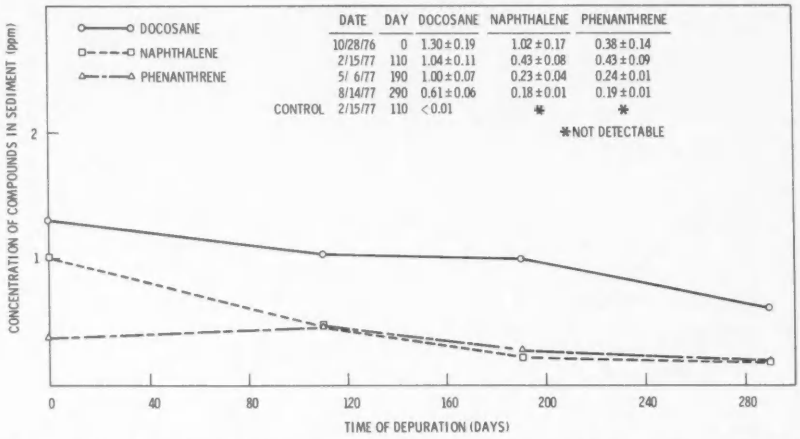



FIG. 6. Concentrations of compound classes in sediments of installation II, as a function of depuration time.

were undetectable in the sediments (Fig. 5). Similar results were observed for the compound classes and total oil concentration (Fig. 6).

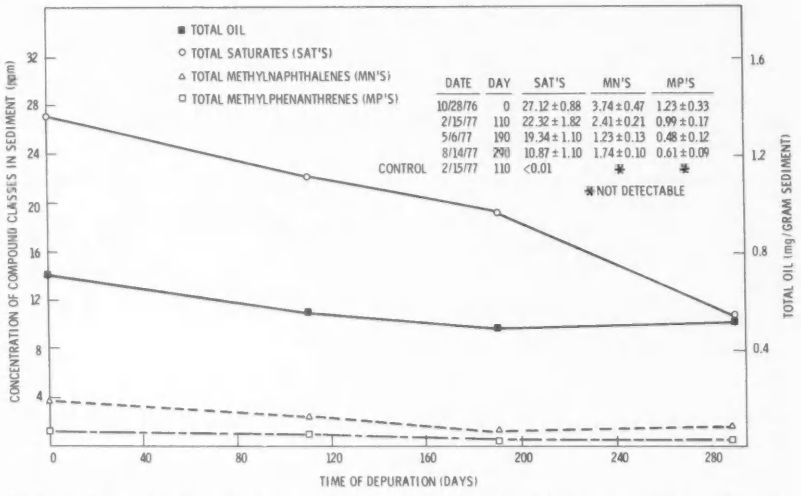
The results of the analyses of installations I and II are in marked contrast to the depuration rates observed in installation III. Initial concentrations of total oil in




| DATE | DAY | DOCOSANE | NAPHTHALENE | PHENANTHRENE |
|---|---|---|---|---|
| 10/28/76 | 0 | 1.30 ± 0.19 | 1.02 ± 0.17 | 0.38 ± 0.14 |
| 2/15/77 | 110 | 1.04 ± 0.11 | 0.43 ± 0.08 | 0.43 ± 0.09 |
| 5/ 6/77 | 190 | 1.00 ± 0.07 | 0.23 ± 0.04 | 0.24 ± 0.01 |
| 8/14/77 | 290 | 0.61 ± 0.06 | 0.18 ± 0.01 | 0.19 ± 0.01 |
| CONTROL 2/15/77 | 110 | < 0.01 | * | * |

*NOT DETECTABLE

FIG. 7. Concentrations of docosane, naphthalene, and phenanthrene in sediment of installation III, as a function of depuration time.




| DATE | DAY | SAT'S | MN'S | MP'S |
|---|---|---|---|---|
| 10/28/76 | 0 | 27.12 ± 0.88 | 3.74 ± 0.47 | 1.23 ± 0.33 |
| 2/15/77 | 110 | 22.32 ± 1.82 | 2.41 ± 0.21 | 0.99 ± 0.17 |
| 5/6/77 | 190 | 19.34 ± 1.10 | 1.23 ± 0.13 | 0.48 ± 0.12 |
| 8/14/77 | 290 | 10.87 ± 1.10 | 1.74 ± 0.10 | 0.61 ± 0.09 |
| CONTROL 2/15/77 | 110 | < 0.01 | * | * |

*NOT DETECTABLE

FIG. 8. Concentrations of compound classes in sediments of installation III, as a function of depuration time.

the sediment were approximately 10% of the concentrations observed in installations I and II. This is reflected in the determined concentrations of individual compounds and compound types (Fig. 7 and 8). After 9 mo 46.9, 17.6, and 50.0% of the original docosane, naphthalene, and phenanthrene, respectively, remained in the sediment (Fig. 7). Greater persistence of saturates (40.1%), methylnaphthalenes (46.5%), and methylphenanthrenes (49.6%) as compound types was also observed (Fig. 8). Total oil analysis indicated 73% of the IR detectable oil remained after 9 mo of exposure (Fig. 8).

The ratios of $nC_{17}$/pristane and $nC_{18}$/phytane were calculated from analysis of the saturate fraction from sediment extracts of field installations I and III. The results are shown in Table 3. In both installations, the ratios remained constant through the period of 4–6 mo. After this, considerable changes occurred in the ratios. In the first installation, the $nC_{17}$/pristane and $nC_{18}$/phytane ratios were lower by factors of 7.3 and 6.4, respectively. The changes observed in the third installation were significant but less than those observed in the first installation. The $nC_{17}$/pristane and $nC_{18}$/phytane ratios were lower by factors of 1.8 and 2.3, respectively.

## BIOLOGICAL

Examination of the list of species identified from

TABLE 3. Ratios of $nC_{17}$/pristane and $nC_{18}$/phytane derived from analysis of sediment extracts from two field installations.

| Date | Days of depuration | $nC_{17}$/pristane | $nC_{18}$/phytane |
|---|---|---|---|
| | | *Installation I* | |
| April 17, 1976 | 0 | 1.59±0.35 | 2.01±0.54 |
| June 21, 1976 | 65 | 1.60±0.39 | 2.01±0.41 |
| Aug. 24, 1976 | 130 | 1.61±1.80 | 2.04±2.32 |
| Nov. 23, 1976 | 221 | 0.22±0.05 | 0.32±0.06 |
| | | *Installation III* | |
| Oct. 28, 1976 | 0 | 1.59±0.03 | 2.21±0.08 |
| Feb. 15, 1977 | 110 | 1.68±0.18 | 2.12±0.31 |
| May 6, 1977 | 190 | 1.46±0.12 | 2.26±0.32 |
| Aug. 14, 1977 | 290 | 0.81±0.10 | 0.98±0.16 |

TABLE 4. Animals found in recruitment trays.

**Phylum Annelida**

Class Polychaeta

Order Errantia
- *Anataides groenlandica*
- *Dorvillea moniloceras*
- *Dorvillea* sp.
- *Eulalia nigrimaculata*
- *Harmothoe* sp.
- *Hemipodus borealis*
- *Lepidonotus caelorus*
- *Lumbrineris* sp.
- *Nothria elegans*
- *Ophiodromus pugettensis*
- *Pholoe minuta*
- *Platynereis bicanaliculata*
- *Typosyllis fasciata*

Order Sedentaria
- *Armandia bioculata*
- *Axiothella rubrocincta*
- *Boccardia* sp.
- *Cirractulus cirratus*
- *Owenia collaris*

**Phylum Mollusca**

Class Bivalve
- *Clinocardium nuttallii*
- *Macoma* sp.
- *Mysella tumida*
- *Mytilus edulis*
- *Protothaca staminea*
- *Psephidia lordi*

Class Gastropoda
- *Acmea* sp.
- *Alabina* sp.
- *Fartulum* sp.
- *Lacuna* sp.
- *Margarites* sp.
- *Nucella lamellosa*

**Phylum Arthropoda**

Class Crustacea

Subclass Cirripedia
- *Balanus* sp.

Subclass Malacostraca
- Order Amphipoda
  - *Corophium* sp.
  - Unknown amphipod
- Order Isopoda
  - *Exosphaeroma amplicauda*
  - *Gnorimosphaeroma oregonensis*
- Order Tanaidacea
  - *Leptocheila* sp.
- Order Cumacea
  - *Cumella* sp.

Subclass Malacostraca
- Order Decapoda
  - *Cancer productus*
  - *Hemigrapsus oregonensis*
  - *Pagurus beringanus*
  - *Pagurus* sp.
  - *Pandalus* sp.
  - *Pinnixa* sp.
  - *Telmessus cheirogonus*
  - *Upogebia pugettensis*
  - *Pugettia gracilis*
  - *Pagurus hirsutiusculus*

field collections (Table 4) shows that the diversity of organisms recruited was quite representative. As might be expected, the dominant phyla were Annelida, Mollusca, and Arthropoda. Because a reasonable portion of the crustaceans may have been transient occupants of a particular sediment tray, these more epibenthic forms were not chosen as good examples of recruited animals. Gastropods were also eliminated from more extensive comparisons between clean and oiled sediment for the same reason. On examination of the numbers of individuals found in each tray, at all or nearly every collection interval, only three polychaetes and two bivalves were shown to be present in significant numbers. We do not feel that selection of five species from the total represents a bias toward oil-resistant species, since these were the only species that were consistently found in control sediments, with one or more individuals per core (in most cases). To determine whether or not the species present within the sediment trays were representive of those found in the surrounding sediment, cores of the outside substrate were taken in September 1977. The polychaete *Armandia bioculata* and the bivalve *Mysella tumida* were present in large numbers (23–39 individuals per core). *Platynereis bicanaliculata* and *Psephidia lordi* were also found to be relatively abundant. Four of the six species used for comparisons between clean and oiled sediment are therefore the dominant infaunal species in the surrounding sediment. Approximately one half of the *Mysella* in the outside samples were found to be mature and this ratio was slightly higher than those observed in the sediment trays.

An overview of the distribution of the two benthic bivalve species is shown in Fig. 9. Installation I contains the largest compilation of data. The trays had been in the field for 385 d by the time the last set of sections was sampled, thus terminating this installation. At the first sampling date (August 24, 1976; 130 d), when the greatest effect from oil in sediment would be expected, larger numbers of individuals were present in the contaminated substrate than in clean sediment. The absence of standard deviations or the large variations (when shown) are due to the fact that entire sections (about 2 L of sediment) were sorted. The numbers of individuals were then converted to numbers present in cores on a volume/volume basis. The only replication was therefore the result of sampling two exposed trays, while only one control tray was analyzed. When replication was possible by use of field collected core samples, variations were still quite large, as shown by the remaining time intervals in installation I. Only in one case was abundance of a species in clean sediment significantly higher than that in exposed substrate, and the difference was very small (*M. tumida* on May 6, 1977). For the bivalves *Mysella* and *Psephidia*, it was possible to discriminate between juvenile and adult organisms. The numbers of mature bivalves increased with time in installation I and at the final interval (385 d), there was a significantly higher number of mature

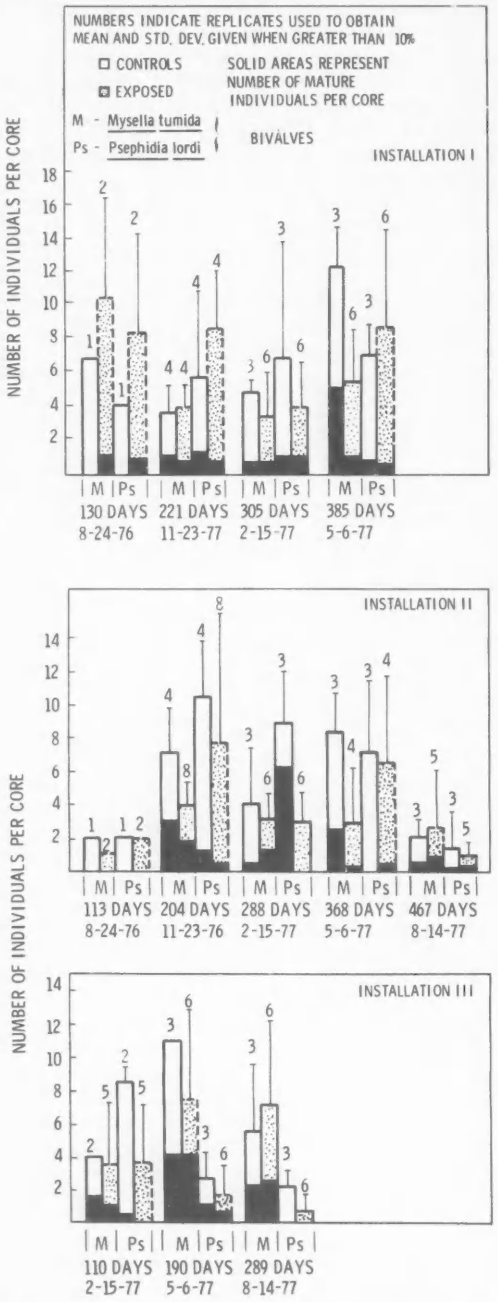



FIG. 9. Relationship between the number of recruited bivalves in sediment cores and time after sediment oiling in three installations. Installations I and II received oil on the surface of coarse sediment (<12 mm), while 0.1% oil was mixed with a finer sediment (<2 mm) in installation III. Height of bars indicates the mean value, with SD and *n* shown above the bars. Stippled bars indicate oil-exposed organisms; solid dark area at the base of vertical bars, the number of mature bivalves.

*Mysella* in the control sediment ($5 \pm 1$ compared with $0.8 \pm 1.1$). This, however, was the only instance of greater abundance of mature bivalves in the clean sediment of installation I.

The conditions of exposure for installation II were exactly the same as I, except that the date of initiation was approximately 3 wk later (May 3, 1976). Only the bivalves from this installation have been identified and counted at the writing of this paper. The results of these analyses are similar to those observed in installation I. Only small differences appear between the abundance of these bivalves at the first two intervals, but *Psephidia* is more abundant in the clean sediment on February 15, 1977. However, it would appear from the data collected on the May and August sampling dates that *Psephidia* in oiled substrate were not eliminated, but are again present in numbers equal to control samples. At the last interval, there are no significant differences between the numbers of mature or juvenile bivalves present in the clean and oiled sediment of installation II.

Installation III was prepared in a manner significantly different than the first two. Sediment was first screened through a 12-mm sieve and then a finer mesh, which allowed only particles <2 mm to pass through. This relatively homogeneous substrate was then thoroughly mixed with oil before being placed in the sediment trays. They provide evidence of the marked difference between the chemistry of these sediments and those of the earlier installations. We noted during field collections that these more homogeneous particles tended to be much more compact, thus making coring more difficult. However, the cores remained intact on extraction and transfer to jars for preservation. It was rather remarkable that this finer, more compact substrate possessed populations of the two bivalve species that were apparently equivalent to those in the other two installations. After only 110 d in the field, reasonably good recruitment had occurred in clean and oiled sediment trays. From this February collection the only significant differences in abundance between clean and oiled substrate were exhibited by *Psephidia*. There were more of the small bivalves (*Psephidia*) in the clean sediment, and a small portion of the population was mature. In May and August there were no significant differences between the populations of the bivalves in clean and oiled substrate, but *Psephidia* tended to be somewhat suppressed in the oil-contaminated sediment. The proportions of mature bivalves in oiled and clean substrate were also very similar.

The populations of polychaetes in installations I and III are shown in Fig. 10. The variability between core samples, even when six samples are available, makes evaluation of effects of oil extremely difficult. There are only two instances in which differences between popula-

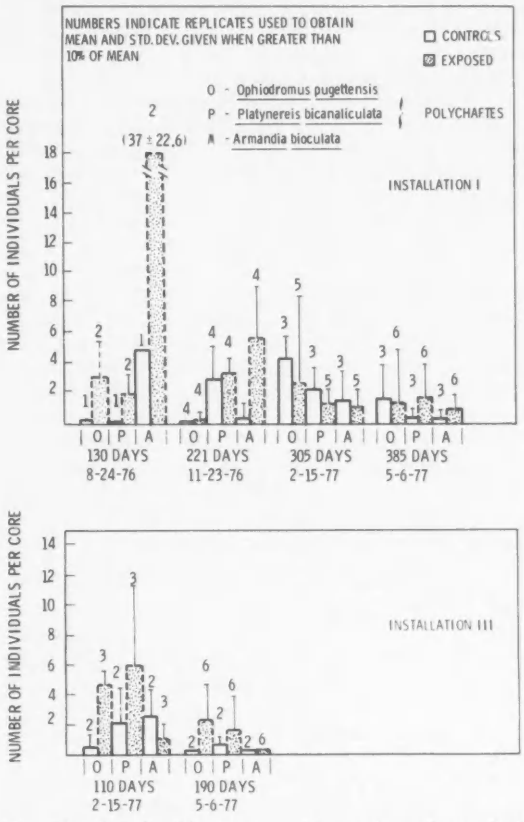



FIG. 10. Relationship between the number of recruited polychaetes in sediment cores and the time after oiling of installations I and III. Height of bars indicates the mean value, with SD and *n* shown above the bars. Stippled bars indicate oil-exposed polychaetes; the open bars, control animals.

tions in clean and oiled substrate are observed, and in both cases the organisms are more abundant in oil-contaminated substrate. The extremely high numbers of *Armandia bioculata* in exposed sediment on August 24, 1976 is a phenomenon not easily explained and not observed again in later collections. Note that at the next collection date (November 23, 1976) *Armandia* was slightly more abundant in oiled sediment than clean substrate, but by February 1977 the populations were approximately equal. Samples from installation III, taken on February 15, 1977, showed that *Ophiodromus pugettensis* was more abundant in exposed substrate than in clean sediment.

In an attempt to correlate season with changes in the abundance of species, the mean values of numbers of individuals were plotted against sampling dates for all three installations. There was no apparent pattern of abundance for four of the five species with regard to

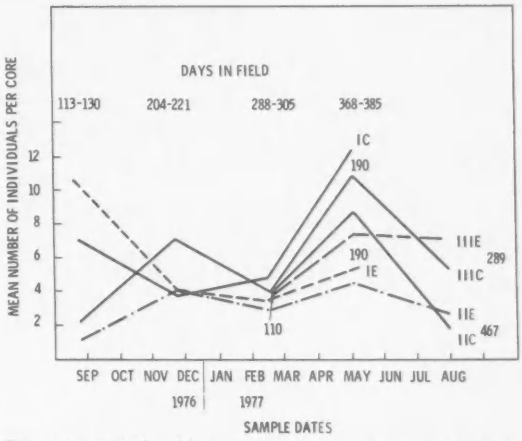



FIG. 11. Relationship between the abundance of *Mysella tumida* in sediment cores of three installations and season. Solid lines represent control (C) sediments; dashed line, exposed (E) substrate. The duration in the field is shown above the time intervals, except for installation III, which had only been in place for 110 d in February, 190 d in May, and 289 d in August 1977.

season or the nature of the substrate. The only trend that was observed is shown in Fig. 11, where the mean numbers of *Mysella* individuals per core are plotted against the calendar. An increase in the numbers of individuals, in all installations from both clean and oiled sediment, is observed for the period February 15–May 6, 1977. This period obviously correlates with increases in day length, air temperature, and water temperature (7–10°C). Populations would be expected to increase during this time, and it is interesting that the means of all exposed collections are lower than the corresponding control populations in May 1977. This pattern is even exhibited by *Mysella* in the third installation, which had been in the field a significantly shorter period of time (110 in February and 190 in May). As noted above, the only significant difference between *Mysella* populations in clean and oiled substrate was that of installation I, and unfortunately, those were the last sections available for sampling. At the last collection date (August 1977) the populations of *Mysella* in installations II and III decrease slightly regardless of contamination level. The abundance of organisms in clean and oiled substrate are not significantly different, and the ratios of mature individuals to the total population are very similar in both types of sediment (Fig. 9).

## Discussion

Considerable differences were observed in the depuration of PBC from the sediment, depending on whether the oil was layered on the surface of the sediment, simulating a spill during calm conditions (installations I and II), or thoroughly mixed into the sediment (installation

III) prior to field exposure, as might be the case during strong storms (West Falmouth spill, 1969). The differences in the rates of depuration of individual hydrocarbons and hydrocarbon types from the two different oil–sediment field installations can be attributed to many physical, chemical, and biological mechanisms. For the sediment that contained a surface layer of oil, tidal and wave action, surface volatility, photochemical and biodegradative processes are more than likely major contributors to the rapid depuration of oil (e.g. installations I and II of the field experiment). In the case of PBC that is thoroughly mixed into the sediment, the slower depuration rates observed can be attributed to some extent to a reduction in the activity of all of the above processes. Changes in $C_{17}$/pristane and $C_{18}$/phytane ratios have been cited as a measure of biodegradation of oil in the environment (Blumer and Sass 1972) and we observed changes in these ratios for installations I and III (Table 2). We suggest that the contribution of the biodegradative process to the depuration of saturate compounds from oil-layered sediment was minimal, because >96% of the oil in the sediment was lost before the biodegradative process apparently began. The greater biodegradative activity for saturate compounds in the layered oil–sediment system can be attributed to the more aerobic environmental conditions expected to be present near the sediment surface. In the mixed oil–sediment system increased rates of depuration are observed after 6 mo for docosane and total saturates (Fig. 7 and 8). The fact that this occurs at the same time that we observed changes in the pristane/phytane ratios suggests that part of this increase in the depuration rate is due to the biodegradative process.

About 7 yr after a Bunker C fuel oil spill (*Arrow*, February 1970) Vandermeulen and Keizer (1977) reported on the persistence of components of this oil in the beach sediment of Chedabucto Bay and the level of contamination in molluscs. They found that bivalve contamination was likely the result of the absence of metabolic pathways to depurate the hydrocarbons. A striking difference between their study of sediment-oil components and our data is evident. Hydrocarbons leaching from tar on the beach to sediments are apparently being selectively retained in the beach sand (Vandermeulen and Keizer 1977). Sand which exhibited considerable contamination from aromatics and cycloalkanes was almost completely free of *n*-alkanes and they propose that selective biodegradation was responsible. Our data do not indicate a preferential degradation of *n*-alkanes, and initial biodegradation was relatively slow. It may be that during the 7 yr of oil leaching, high populations of oil-degrading microorganisms have been established at their study site, and we are just beginning to see an indication of this process after about 7 mo.

Results of biota analyses are not as definitive as chemical determinations, a fact we are sure that is not surprising to biologists. The variability among core samples in a given sediment tray and among collection dates precludes absolute evaluation of effects on recruitment. While a large number of species was represented at least once in a sample, only five were present in sufficient numbers and frequency for comparisons to be made between populations in clean and oiled substrate. Wide fluctuations in the seasonal abundance of several species have made it necessary to eliminate most organisms from consideration. Control sediment has never shown any indication of "carry-over" contamination, so the absence of most species in clean sediment on one or more collection dates is not the result of oil in the environment.

When comparing the abundance of the two bivalve and three polychaete species, few significant differences between populations in clean and oiled substrate were observed. While the populations of the bivalve *M. tumida* were depressed in all oiled substrates (all three installations) in May 1977, the August collections for installations II and III did not exhibit this pattern. *Psephidia* in installation III seemed to be consistently depressed in oiled substrate, but the differences are not always statistically significant. The seasonal effect (spring increase) on abundance of *Mysella* was consistent for all three installations even though the method of oil application and the duration of depuration for installation III was quite different. From chemical data the levels of total hydrocarbons in the oiled sediments of installations I and II were 100 ppm or less, while those of installation III still contained 490 ppm on May 6, 1977. It is most interesting that this latter concentration did not produce a greater suppression of the population of *Mysella*, as well as other species during the spring.

When comparing this investigation with others on the fate and effects of petroleum hydrocarbons in the marine environment, one must either consider chemical studies or biological studies, as others have generally not used a "team" approach in field experiments or spill studies. Most field studies have been conducted after spills, when baseline data and/or a suitable control site are not available for comparison of pre- and postspill conditions. There are, however, a few relatively recent studies that present data that may be compared with those of this investigation.

An extensive field study has been described by McAuliffe et al. (1975) that concerned the effects of a 65 000 barrel oil spill, 18 km east of the Mississippi River delta on the continental shelf. They reported that oil in sediment was generally restricted to a 17-km radius from the platform and that linear regression coefficients did not show significant correlation between any of the biological parameters measured (number of species, number of individuals, diversity index, and crustacean/polychaete ratio) and the amounts of either $C_{12}$–$C_{23}$ or $C_{12}$+ hydrocarbons. It was estimated that 0.4–0.5% of the total oil discharged was present on the sediments within a 8-km radius of the platform. A value of about 130 ppm total hydrocarbons (measured gravimetrically) represented the contaminated area and could be compared with the IR values for the exposed

sediments in this study, which were initially as high as 770 ppm.

Using total organic carbon as a measure of the extent of oil contamination, Wormald (1976) studied the devastation and later recovery of meiofauna in beach sediments of Picnic Bay, Hong Kong, which received a spill of heavy marine diesel oil. He showed that nematodes and harpacticoid copepods were almost completely killed within 4 d after the spill and populations remained very low for 8 mo. Factors that were apparently controlling the rate of recovery (recruitment) were total oil levels, percent of aromatics, and position on the tide level. The latter factor seemed to be important because of physical–chemical and perhaps microbiological activities which in turn affected the retention time of oil in the sediment. Nematode populations recovered before those of harpacticoids. About 11 mo were required to reduce hydrocarbon concentrations in sediment from about 1% (about 10 000 ppm) to background, and after 15 mo the meiofauna were present at "normal" population levels.

Another investigation of the effects of spilled oil was recently reported by Krebs and Burns (1977), in which they described the gradual recovery of fiddler crab, *Uca pugnax*, populations corresponding to concentrations of weathered No. 2 fuel oil in sediments. Recovery is still not complete 7 yr after the spill, and concentrations in the mud >1000 ppm were toxic to adults, while those of 100–200 ppm were toxic to juveniles. Abnormalities related to sublethal chronic exposures were also found, and it appears that this species may not have the capability to metabolize hydrocarbons exhibited by other crustaceans (see review by Lee 1977).

## Conclusions

The rates of depuration of hydrocarbons in the two installations of coarse sediments that received a surface oiling were quite rapid and very similar. When we consider the methylnaphthalenes (MNs) and methylphenanthrenes (MPs) which have been shown to be as toxic as any compounds tested (Neff et al. 1976), the concentrations in the sediments (initially 25–30 ppm MNs and 12 ppm MPs) decreased by nearly an order of magnitude in 100 d and were not detectable after 200 d. On the other hand, when a smaller amount of oil was mixed into the sediment, concentrations (initially 4 ppm MNs and 1 ppm MPs) only decreased by a factor of 2 or less in 100 d and detectable levels were still present after 290 d.

The hydrocarbon contamination in all three installations was therefore very similar after the first 100 d of depuration in the field (2–4 ppm MNs and 1–3 ppm MPs). This factor perhaps helps to explain the rather similar results obtained in benthic organism recruitment. It is still surprising that a greater inhibition of recruitment did not occur, when initial concentrations of hydrocarbons were quite high and levels after 100 d would appear to be deleterious. For comparison, concentrations of total naphthalenes and phenanthrenes in the initial sediments of installation I were 110 and 30 ppm, respectively, and MacLeod et al. (1977) showed that a highly contaminated sediment contained about 0.2–1 ppm of these components. Concentrations in our experimental sediments were apparently well above those encountered in even a highly polluted substrate.

With the approach used in this study, it is still possible to follow variations in populations over many more months to observe long-term effects. The apparent depression in bivalve populations may prove to be significant at later intervals. It is indeed difficult or impossible to compare the effects (or lack of them) discussed in this study with those of other field investigations where data on the composition of hydrocarbon contamination are not described. Armstrong et al. (1977) found that the levels in chronically contaminated mud near a separator platform contained 12.4 ppm total naphthalenes and 2.2 ppm total phenanthrenes and that the benthos was depressed. Based on these findings, one might expect to find the recruitment measured in this study to be affected by the oil. These differences in results are likely due to the duration of exposure and the numerous variations in environmental factors which have made comparisons between spill studies so difficult.

We have clearly demonstrated the rates of depuration of individual hydrocarbons and hydrocarbon classes after different types of oil introduction to sediment. Obviously, the composition of the substrate markedly influences the rate of oil depuration and the effects on biota. To date, the longest duration of oil effects on benthic populations has been 7 yr, as recently reported by Michael (1977) and Michael et al. (1975). The type of oil, means of introduction, and habitat affected at West Falmouth, Mass., would seem to be the worst possible combination for damaging the ecosystem. Our studies indicate that based on hydrocarbon depuration from coarse sediment, the recovery rate of a typical intertidal zone in the Pacific Northwest would be much more rapid. Initial results from benthic organism recruitment studies indicate minimal effects from oil application. However, more thorough analyses of organisms and continued sampling must be conducted before a definitive conclusion can be reached.

## Acknowledgments

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*SESSION III. Summary and Overview*

# Long-Term Effects of Oil Spills on Marine Intertidal Communities

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OIL pollution may cause massive local destruction of marine plants and animals as a result of shipping accidents, oil well blowouts, pipeline failures, and the like. Low-level but continuing pollution, as from refinery effluents, may cause progressive environmental deterioration which may be slower to appear but is no less destructive than spectacular accidents. As this symposium has shown, there is a solid body of information about the direct effects of these types of oil pollution. Except for the losses of pelagic seabirds, spilled oil appears to cause measurable biological damage only when it is in very shallow water or is stranded on the shoreline. More important than the initial damage caused by oil pollution is the length of time the environment remains in a damaged state, and we have more fragmentary information about this. Much less is known about the consequences of petroleum hydrocarbons entering food webs in the sea, where they might behave as conservative, long-lived contaminants and have a long-term debilitating effect on marine organisms.

At present, attention is focused on the conspicuous consequences of oil pollution. They do not differ in principle from other, natural and man-made perturbations of the marine environment and to set them in perspective, it is useful to view them in the context of the broader aspects of ecology.

## Potential for Recovery

The title of this symposium is *Recovery Potential of Oiled Marine Northern Environments*. It is clear from the case histories of oil spills reported here that the potential for recovery is indeed present. In most cases, within 10 yr of a single incident the community structure has returned to something approaching its normal state. The rate of recovery depends on many factors. One is the toxicity and quantity of oil involved; light fuel oil is much more toxic than crude oil or bunker oil and causes much greater environmental damage. Another is the nature of the shoreline on which the oil is stranded; on a high-energy, rocky shore the oil is dispersed and removed by wave action much more quickly than on a sheltered, sedimented site, where the oil may become incorporated in sediments, persist for many years, and cause chronic perturbation to the ecosystem. A third is the biology of the organisms that have been affected. Organisms with little or no mobility and slow rates of reproduction will take much longer to recolonize a damaged area than species that produce large numbers of young that are widely dispersed. Since the former is a characteristic reproductive pattern for many organisms living in high latitudes, recovery of damaged arctic environments may be much slower than that of the temperate and subboreal environments that were the subject of these investigations.

There is, of course, a danger that the documented presence of a recovery potential may lead to a lack of concern on the part of those responsible for prevention of oil pollution or cleanup. We have two observations to make on this point. First, recovery cannot begin until the polluting substances have been removed. For small spillages under exceptionally favorable circumstances this may occur naturally, but generally it requires a major human effort to remove the oil. In all the incidents discussed in this symposium, the recovery that was documented would not have taken place if it were not for the effort to clean up the spilled oil Second, although we can say that many sites will show substantial recovery in 10 yr, this is a very long time to be deprived of the amenities of a pleasant shoreline for those who live in the area. There is no room at all for complacency or for the view that oil spills are unimportant forms of environmental deterioration.

## Variability in the Marine Environment

A problem that arose several times during the presentation of papers was in relation to "controls," "standards," or "reference sites." It is natural that those who document changes in the biology of particular organisms at the site of an oil spill should select one or more unoiled sites in the neighborhood for the purposes of comparison. They are necessary as a guide to the extent of the damage caused by the oil and as a measure of the "normality" to which the damaged environment returns during the recovery phase. Some authors referred to them as "controls"; others, in response to questioning, agreed that the unoiled sites were really quite "uncontrolled" and that "reference site" might be a better term. We believe that there is a basic problem here, which deserves further exploration and will need ex-

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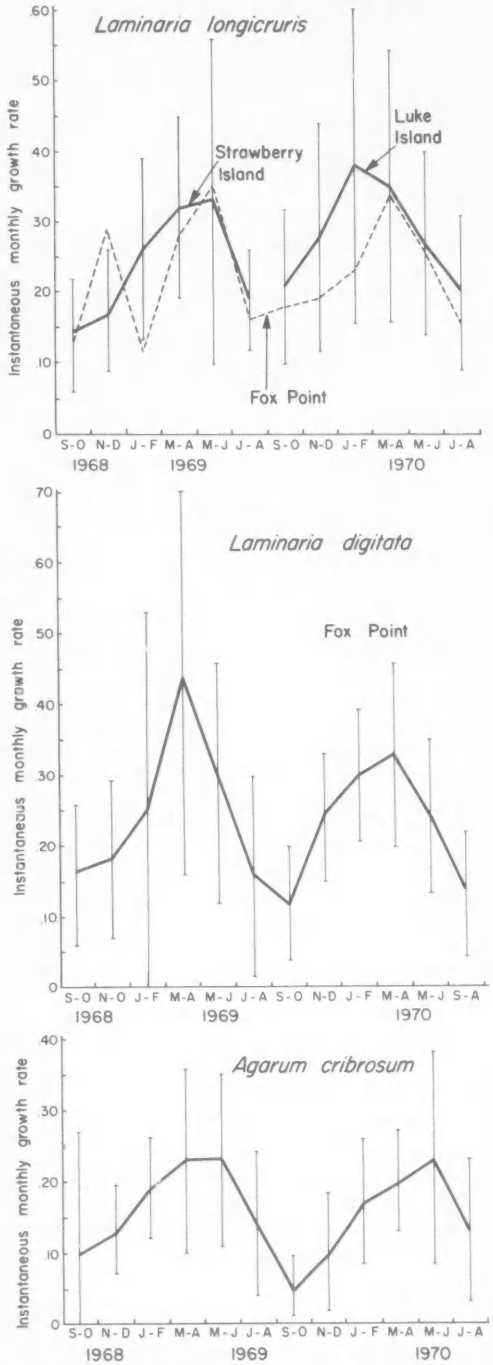
Laminaria longicruris
Strawberry Island
Luke Island
Fox Point
Instantaneous monthly growth rate
S-O N-D J-F M-A M-J J-A S-O N-D J-F M-A M-J J-A
1968 1969 1970
Laminaria digitata
Fox Point
Instantaneous monthly growth rate
S-O N-O J-F M-A M-J J-A S-O N-D J-F M-A M-J S-A
1968 1969 1970
Agarum cribrosum
Instantaneous monthly growth rate
S-O N-D J-F M-A M-J J-A S-O N-D J-F M-A M-J J-A
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FIG. 1. Seasonal growth patterns of seaweeds in eastern Canada. From Mann (1972b).

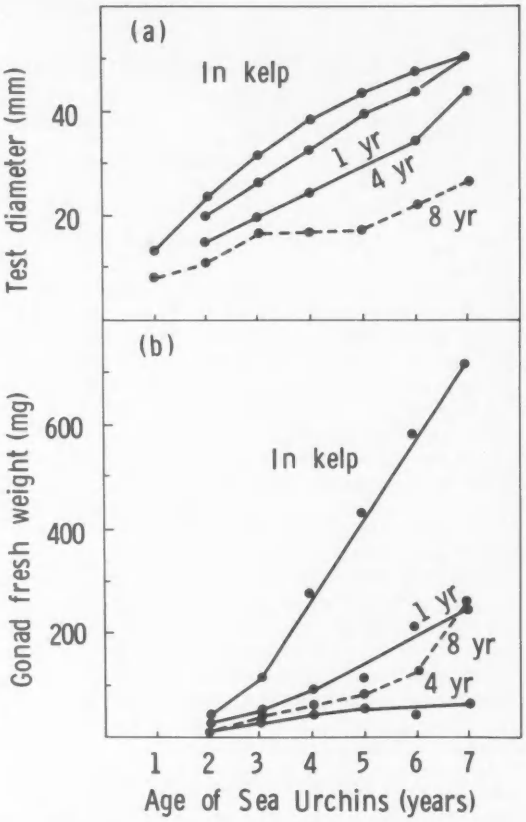


FIG. 2. Growth curves and gonad production curves for the sea urchin *Strongylocentrotus droebachiensis* in St. Margaret's Bay, N.S. The urchins were collected from healthy kelp beds and from sites where kelp had been destroyed by overgrazing 1, 4, and 8 yr earlier. From Mann (1977).

plicit recognition. It is, that organisms in natural, unpolluted marine ecosystems are inherently extremely variable, and establishment of what is "normal" is a much more difficult task than most people realize.

To establish this point, we propose to review some examples from unpolluted sites. The first will be from St. Margaret's Bay, N.S. In a survey of the marine algae it was found that dense beds extended far out from shore, to a depth of about 20 m, and that the intertidal algae constituted $<10\%$ of the biomass (Mann 1972a). In passing, we may note at once that from the point of view of the total system, destruction of the algal beds between tidemarks by an oil spill would not be total disaster; it would be loss of $<10\%$ of the biomass. However, from the point of view of this discussion the most important finding was that when we studied the growth rates of hundreds of individual plants in different parts

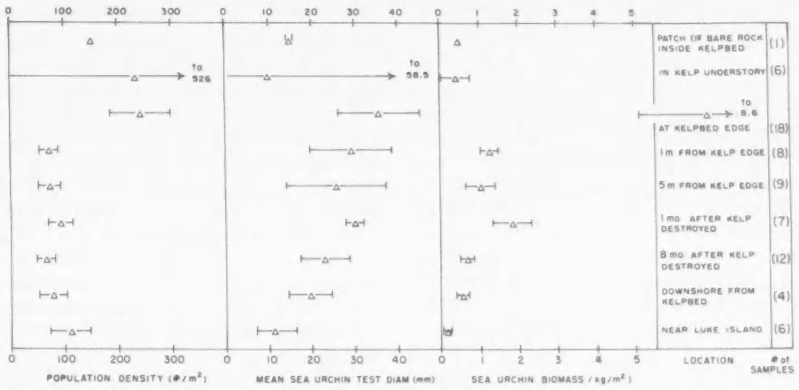
PATCH OR BARE ROCK INSIDE KELPBED (1)
IN KELP UNDERSTORY (6)
AT KELPBED EDGE (18)
1m FROM KELP EDGE (8)
5m FROM KELP EDGE (9)
1 mo. AFTER KELP DESTROYED (7)
8 mo. AFTER KELP DESTROYED (12)
DOWNSHORE FROM KELPBED (4)
NEAR LUKE ISLAND (6)
to 926
to 58.5
to 8.6
POPULATION DENSITY (#/m²)
MEAN SEA URCHIN TEST DIAM (mm)
SEA URCHIN BIOMASS (kg/m²)
LOCATION
# of SAMPLES

FIG. 3. Population density, biomass, and mean test diameter of sea urchins observed at various locations in St. Margaret's Bay, N.S. Bars indicate 2 SE for density and biomass; 2 SD for mean test diameter. From Breen and Mann (1976).

of the bay (Mann 1972b), we found that the standard deviations of the growth rates were very large, up to ±100% of the mean value (Fig. 1).

The most important consumer of these large algae, or kelps, is a sea urchin (*Strongylocentrotus droebachiensis*). During the past 10 yr we have found that these urchins can form dense aggregations and totally destroy the large algae over a considerable area. They then remain in that same area, attached to almost bare rock, and survive at least 8 yr with steadily declining growth rates. In Fig. 2 the growth curves are given for urchins that have been on bare rock for 1, 4, and 8 yr. It can be seen that the growth rate after 8 yr is about half what it was when kelp was present. In the face of this kind of variability it would be extremely difficult to distinguish an effect due to the presence of oil. Similar remarks can be made about sea urchin biomass (Fig. 3). In various sites, all within St. Margaret's Bay, population density varied by a factor of 10 (Breen and Mann 1976). We believe that the perturbation giving rise to this variability was removal of predators of the sea urchins, especially lobsters. However, Dayton (1971) has documented comparable cycles of change in intertidal communities which were begun by the impact of floating logs on a rocky shore.

The view is gaining ground that not only is this kind of variability being generated all the time by chance events which have repercussions through the system, but that individual species, as part of their strategy for survival in the marine environment, have high levels of variability built into their gene pools. Chapman (1974) showed that populations of the kelp *Laminaria* have tremendous variation in the size and structure of their stipe (or stalk) and although some of this is a phenotypic response to water movement, there is strong genetic selection for different kinds of plants at different sites. Similarly, Doyle (1974, 1975) showed that there are major genetic differences in populations of the tube worm *Spirorbis* between exposed coast, open tide pools, and sheltered tide pools, separated by only a few hundred metres. Oysters from different sites have been shown to have genetic differences in growth rates and salinity tolerances (Newkirk et al. 1977), and periwinkle populations in different areas show genetic differentiation in respect of shell shape, which in turn is related to the ability to withstand wave action and desiccation (Newkirk and Doyle 1975).

Hence, those seeking to document the effects of oil pollution on marine organisms are faced with a severe practical problem: how to obtain enough data on the natural variability of the organisms to establish that those exposed to oil are significantly different. Dr Sanders, in particular, spoke with some feeling about the difficulty of obtaining the funding necessary to get samples with sufficient replication, and over a sufficiently long period, to properly establish the effect of an oil spill. Our suggestion, as a contribution to resolving the dilemma, is that it pays to shift attention from single species to whole systems. The papers that showed what effect an oil spill had had on a total community seemed to us more convincing than those that documented effects on specific organisms in the field. By shifting emphasis to the total community of interacting species, variabilities of individual components of a system are evened out, and properties of the total system are seen to change in meaningful ways. Dr Southward's account of changes in the intertidal communities of Cornwall following the *Torrey Canyon* disaster appears to be a good example of this approach.

## SOME EXAMPLES OF THE ECOSYSTEM APPROACH

Ecosystems, or total biological communities in interaction with their environments, are daunting in their complexity and therefore extremely difficult to document by precise measurements. As we have seen, studies

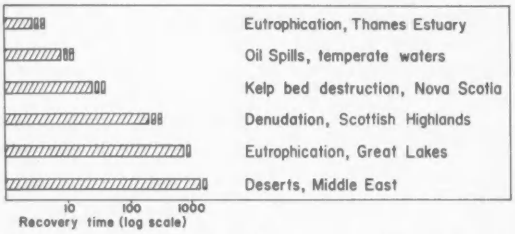


FIG. 4. Estimates of possible recovery times (on a log scale) for various environmental perturbations. Estimates are of the time taken for the system to recover after the cessation of the activity causing the perturbation.

that focus on individual components tend to be unsatisfactory because those components vary in response to apparently unrelated perturbations in other parts of the system. For practical purposes we need good indices of the integrated performance of ecosystems. An index that is widely used is species diversity. Although it has been widely criticized, we think it has emerged as a valuable tool. Dr Sanders' paper in this symposium showed very clearly how stresses such as oil pollution are correlated with lowered diversity indices. Another index, more difficult to measure but well-worth exploring, is productivity at various trophic levels. There is now an enormous body of data on the productivity of various marine systems, and there is a possibility that oil pollution has predictable effects on productivity. Some kinds of secondary productivity may be enhanced if the possibility exists of organisms using oil hydrocarbons as a source of energy and materials. In other cases, where plants are killed by the pollutant, it would be valuable to know what percentage of the total primary productivity of a system has been eliminated.

In this context, we are impressed with the paucity of information that exists on the functioning of arctic and antarctic ecosystems, so that it is possible for informed people to have widely differing views on the probable effects of an oil spill under arctic ice. We know, for example, that the same species of large seaweed that occurs along the Nova Scotia coast is abundant in arctic waters, yet in the latter situation it may live in almost total darkness for 6 mo of every year. What effect does this have on its vulnerability to oil pollution?

## Capacity for Assimilating Oil

Dr Pallister, in his excellent lunchtime address to this symposium, asked whether it would be possible to quantify the ability of the marine environment to assimilate oil. As with so many kinds of pollution, the industrial world hopes for clear guidelines: this much pollution can be assimilated without harmful effects; above a certain figure the effects are undesirable. We have no ready answers. In ecosystem terms, assimilation means conversion to carbon dioxide and water. The rate at which this proceeds is very variable according to the numbers and kinds of microorganisms present, the availability of nutrients other than hydrocarbons, and of course, the prevailing temperature. Even if we were able to quantify these factors, and hence estimate the rate at which certain kinds of oil hydrocarbons are metabolized, we would have to face the difficult question of whether some of the compounds are resisting attack, and may be accumulating in the food webs. We found Dr Stegeman's exposition on mixed-function oxygenases to be particularly illuminating in relation to this question. This is an area in which further work is badly needed. In the present state of knowledge, our best index of an "acceptable" level of oil pollution, in relation to a particular ecosystem, might be a level at which the ecosystem appears to be functioning normally and there is no food-web magnification of substances potentially harmful to the organisms or man.

## Oil Pollution in Relation to Other Man-Made Perturbations

Those whose job it is to find the resources for environmental protection have every right to ask the questions: How serious a problem is oil pollution in the marine environment? What priority should we give it in relation to other forms of pollution, or in comparison with other problems of environmental degradation? Again, we have no instant answers but as an aid to rational judgement we offer the suggestion that the concept of recovery time might be one index of the seriousness of a man-made environmental degradation. Figure 4 gives our rough subjective impression of the time scale involved in the recovery of different degraded ecosystems in the biosphere. The reasoning behind each estimate is as follows. The deserts of the Middle East, which appear to have arisen from chronic overgrazing in an area of seasonally low rainfall, have reached a stage where natural productivity is extremely low and the carbon and nutrient content of the soils is also extremely low. If grazing could somehow be stopped tomorrow it seems probable that a natural process of return to the high productivity recorded in ancient times might well take in excess of a thousand years. A similar, but less severe case of reduced terrestrial productivity occurred a few hundred years ago in the Scottish Highlands, when the productive natural forests and other forms of vegetation were cleared to make way for grazing. The consequent soil erosion and leaching has led to a form of man-made desert, less severe than the first example, and thought likely to be capable of regeneration in a few hundred years if grazing could be suppressed.

Another kind of man-made perturbation, more recent in human history, is enrichment or eutrophication of water bodies. Addition of nutrient materials, especially phosphates, leads to a great acceleration of the process of carbon reduction (in photosynthesis) after which completion of the cycle of death and decay leads to oxygen deficits in the water and major changes in the whole

flora and fauna. The Great Lakes, which have undergone such changes, now have such enormous deposits of nutrient-rich sediments that even if the influx of nutrients were to stop tomorrow, return to the earlier state might be expected to take many hundreds of years. On the other hand, enrichment of the Thames estuary by sewage effluents from London led to a condition that was readily reversible. After improvement in the sewage treatment facilities, there was a marked recovery in the estuary within a period of 5 yr. Clearly, the difference between the two situations is a function of the flushing times involved.

Intermediate in recovery time is the situation described earlier, where removal of a key predator (the lobster) has led to aggregations of sea urchins and destruction of underwater kelp forests. Highly productive, once dense beds of *Laminaria* and *Agarum* have been eliminated over many hundreds of square kilometres of coastline in eastern Canada. By comparison, the damage by oiling of 1 or 2 $km^2$ of salt marsh is a minor loss of marine productivity. (Aesthetically it is of course much more visible and hence more disturbing.) We have observed areas of "urchin-dominated barren grounds" since 1968, and only now, 9 yr later, can we see the first signs of possible kelp regeneration. The kelps have effective spore dispersal over a very limited area, and we believe that if fishing of lobsters stopped tomorrow it would take 10–20 yr for the system to regain full productivity.

Against this background, an oiled marine system with a recovery time of the order of 10 yr is seen as being far from the worst kind of environmental perturbation that can be envisaged. However, recovery time for the local ecosystem after an isolated incident is only one measure of the seriousness of a situation. We have heard accounts of areas subjected to recurring inundation with oil and of areas vulnerable to the synergistic effects of several pollutants. Furthermore, in areas of chronic low-level oil pollution the activities of mixed function oxygenases in various organisms may lead to the production and food-web accumulation of potentially toxic substances.

## Conclusions

Our strongest conclusion is that for an adequate understanding of the effect of oil on marine systems, we need much more work directed at the properties of the natural systems. This is a problem that is at the heart of environmental protection and that is difficult to solve under the present science policy arrangements. Agencies concerned with urgent practical problems understandably look for useful answers on a short time scale. Unfortunately, the marine ecosystems we wish to protect are themselves so complex and their components so richly interconnected that only large-scale sampling programs and long series of observations show meaningful results.

Detailed studies of processes within the systems, such as microbial degradation of oil, tissue retention of hydrocarbons, and effects of hydrocarbons on the physiology of particular species, or on their population dynamics, are useful pieces of the jigsaw puzzle, and should be pursued with all vigor. Many good examples of this type of study were reported at the Symposium, but the number of good studies at the ecosystem level is very small and needs to be enhanced.

We concluded that while an oil spill is not by any means the most long-lasting kind of environmental perturbation, it is none the less far too frequent, and recovery of the natural systems depends first and foremost on very effective arrangements for cleaning up the spilled oil. Since this is a very expensive process, sometimes costing millions of dollars, it is likely to make economic as well as ecological sense to take every possible step to avoid the spills in the first place.

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